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THE PERIODIC MOVEMENTS OF THE PRIMARY LEAVES  
OF CANAVALIA ENSIFORMIS

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THE PERIODIC MOVEMENTS OF THE PRIMARY  
LEAVES OF THE SPROUTS OF  
CANAVALIA ENSIFORMIS

Doctoral Thesis in Physics and Mathematics written for  
the State University, Utrecht, under Rector Magnificus,  
Professor J. Ph. Suyling, of the Faculty of Jurisprudence,  
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the Faculty of Physics and Mathematics, to be defended on  
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Gerrit Brouwer,  
of The Hague

ABSTRACT. Periodic movements of the primary leaves of the jackbean (*Canavalia ensiformis*) are investigated. After a discussion of previous investigations in this field, personal investigations are described. Included in the experiments are tests involving normal curves, artificial and covered leaf blades, cut leaves, submerged leaves, and leaves with removed nodes, as well as tests employing differing periods of constant and alternating light and darkness. The hypothesis that an unknown factor in the atmosphere affects the nodes of plants, which induce turgor changes causing periodic movements in the leaves is presented and defended. Results of clinostat tests are examined, and main conclusions are summarized.

I am happy to take the opportunity to stop for a moment at the completion of this thesis, and to look back at the pleasant time that is behind me. Although I was never able to fully enjoy a student's life for financial reasons, these years made an unforgettable impression on me, so that I regard them as a great privilege. I recall all who have contributed to my scientific education with great gratitude.

Professor Nierstrasz, your colleagues have always seen be because you know how to distinguish very clearly main things from matters of minor importance, whereby great problems came so distinctly to light.

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From you, Professor Jordan, I have learned how useful it is to keep the problems distinctly in view during a scientific investigation. Much that I have learned from you I was able to apply with a great deal of advantage in the instruction of my students.

Professor Westerdijk, the times I was able to spend working in your laboratory under your inspiring guidance will remain unforgettable. Conversations with you, the walks through the beautiful Canton Park in Baarn, and the instructive excursions which we made to different nurseries under your guidance are delightful memories.

I transferred the interest which you awakened in me for fungi and bacteria to my students, and in this way was able to tell about the enormous and important work which is carried out in the Phytopathological Laboratory.

Professor Dr. van Iterson, I am unable to express my great gratitude to you in just a few words. You granted me hospitality in your laboratory in the most perfect manner. For two years I was able to use the dark chamber in your



laboratory and the experimental greenhouse. You put at my disposal all possible means, and also stood at my side with your vast knowledge, and, during moments of discouragement, you were able to inspire me with new zeal. Without your help and cooperation I never could have developed this subject for my dissertation.

Professor Went, esteemed sponsor of my degree, I consider it a great honor that you were willing to be my sponsor. However busy and occupied you were as a scientist of international fame, you always had time to discuss study interests or particular subjects with your students.

The special atmosphere created by you and your wife left an indelible impression on me.

You taught us how useful strict veracity and severe criticism are, not only in reviewing the treatises of other research workers, but, above all, in applying them to the results of own experiments. I shall never forget the wonderful time I spent in your laboratory.

Work on this thesis would not have been possible for me without the collaboration and assistance I received from the directors of the two high schools with which I was connected. Esteemed Messrs. Kamerbeek and Dr. van der Wijk, due to your sympathy, my 23 lessons were so arranged that I had as much free time as possible, while substitute hours were as few as possible. My heartiest thanks to you for your cooperation.

Finally, I am grateful for all the help I received from personnel of the Culture Garden for Technical Crops and of the Laboratory of Technical Botany in Delft.

Mr. A. de Bouter, draftsman of the Botanical Laboratory in Utrecht, was very kind to draw all illustrations for me, for which I am very grateful.

I also want to thank the Assistant of the Botanical Laboratory in Utrecht, Mr. P. A. de Bouter, for the excellent manner in which he arranged the dark chamber for my tests at such short notice.

## PREFACE

This investigation initially started with a number of tests I made in the Botanical Laboratory in Utrecht in 1922.

I chose this subject for my dissertation on the advice of Prof. Dr. F. A. F. C. Went.

However, because of my job in secondary education at The Hague, it was impossible for me to complete the investigation at Utrecht.

Prof. Dr. G. van Iterson, Jr., however, was very kind to let me work at the Laboratory of Technical Botany in Delft, and I accepted this opportunity with the approval of Prof. Went.

In the summer of 1925 I repeated a number of tests, which I had made in Delft, in the Utrecht Laboratory for comparison.

## TABLE OF CONTENTS

Abstract .....	ii
Foreword .....	iii
Preface .....	iv
CHAPTER I. Review of Literature .....	2
CHAPTER II. Personal Investigations .....	31
A. Method of Procedure .....	31
B. Tests in the Greenhouse .....	39
1. Discussion of normal curves .....	39
2. Tests with an artificial leaf and with a covered blade .....	42
3. Tests with cut leaves .....	45
4. Tests with leaves under water .....	52
5. Removal of the upper or lower half of the node .....	53
C. Tests in the Dark Chamber .....	57
1. The movements of plants raised in constant light .....	57
2. Introduction of a 24-hour period of light and a 24-hour period of darkness .....	61
3. Tests with stems .....	63
4. Reversal of the normal period .....	67
5. Tests in constant darkness .....	73
6. Tests in constant light .....	77
CHAPTER III. Theoretical Considerations .....	82
CHAPTER IV. Clinostat Tests .....	90
Review of the Main Results .....	92
References .....	96

\*Plate 1. P17 (left) with stalk weight.  
P17' (right) without stalk weight.  
12 December 1924, 2:30 P.M.

Plate 2. P17. After removal of stalk weight.  
16 December 1924, 2:30 P.M.

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\* Translator's Note: Figures are not reproducible.

## CHAPTER I

### Review of the Literature

We can easily observe the phenomenon that leaves and flowers assume different positions in the evening than during the day. The compound leaves of acacia, for instance, fold up their leaflets at this time upwards against each other. On a field of beans (*Phaseolus spec.*), we can also see that toward the evening the leaflets are starting to droop. Moreover, movements are often carried out by stalks.

/1\*

Many flowers, such as tulips, crocus, and many composites, close up in the evening, and reopen in the morning.

It became evident that in some cases the cause is the transition of light into darkness, or a temperature change. So, the direction of the light has no influence here.

Therefore, we do not speak of tropism, but of a nastic movement. And because the change of day and night plays such a great role here, we speak of a nyctinasty.

Now then, the movement of the organ relating to this could be caused by an uneven growth of the upper and lower side, or a different turgor of the two sides. In the first case, we speak of a growth or nutaton movement; in the second case, we speak of a variation movement.

The nutation movement ceases, of course, as soon as the organ is fully developed; the variation movement, on the other hand, continues. Here, almost always the movements are carried out by special organs, the "joints."

This is clearly demonstrated on the leaves and leaflets of many Leguminosae and Oxalidaceae (Handgirg) [17].

We find nutation movements in Alsineae, Compositae, Solaneae, and Balsaminaceae, among others (Batalin) [2].

All these movements are caused by external stimulation, such as change in light or in temperature. Therefore, we take them together rightly as para- tonic movements, as compared to spontaneous or autonomous movements that are (apparently) independent from the external environment, but are supposed to

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\* Numbers in the margin indicate pagination in the foreign text.

be caused by internal factors. A well-known example of this is *Desmodium gyrans*, the leaflets of which make constantly revolving movements.

We will now pass on to an approximately chronological discussion of the articles which have a bearing on this subject.

It is not amazing that Plinius [33] had already pointed out the phenomenon of leaf movement. He saw that leaflets of clover started to droop in an on-coming thunderstorm. Quite likely he had also known the daily sleep movements.

Yet it was not until 1751 that Linnaeus [24] named this phenomenon "plant sleep."

Thereafter, various authors tried to set up a theory to explain this peculiar behavior. With the aid of a piece of cardboard upon which a 90° angle divided into degrees was drawn, the position of the leaf with respect to the vertical is measured more or less many times a day. These points are then plotted on paper and connected by a straight line. Thus, we obtain in outline a general view of the movements made. It was not until 1907 that Pfeffer used the self-recording method.

Hill [18] was the first who announced, in 1757, that the daily variation of light and darkness was the cause of sleep movements.

Zinn [56] (1759) could not confirm this by his tests, and therefore assumed internal factors as causes.

In 1806, De Candolle [10] made tests with artificial illumination, and from these he concluded that changing illumination is of the greatest importance in the development of sleep movements. However, he could not definitely establish whether the light itself was the initiating cause of nyctinasty, or just hereditary characteristics fixed in the plant to carry out periodic movements arranged in a certain manner.

In 1837, Dutrochet [14] came to the conclusion that light has a double significance for the occurrence of these movements. Whenever, as a result of constant darkness, a rigidity ("darkness rigidity") develops, and then light is provided again, this is supposed to make the sensitiveness return, while, in addition, it is also the cause of movement. A decrease in this sensitiveness in the evening is supposed to cause the sleep condition.

Dassen [13] (1837) seems to assume a cause of movement fixed by heredity,

/3

while being heavily influenced by external stimuli. It is also peculiar that, according to this author, light should have very little influence, while heat and humidity should exercise a very great influence.

Meyer [25] was successful in 1839 in reversing the daily cycle by artificial illumination so that the leaves were in a daylight position in the evening, and drooped in the morning hours.

Brucke [9] (1848) thought that the movement carried out as a consequence of stimulation by light or heat may be similar in its expression to the daily sleep movement, but that it is still different by nature.

Sachs [35] found in 1857 that the function of the node is such that when one half of it shows an increase in the turgor, the other half will have a decrease of pressure.

Later (1863) [36] he came to the conclusion that plants have an ability of movement that is established by heredity, which, however, should occur in definite regularity by alternating illumination.

Hofmeister [19] (1862) agreed with the ideas of Sachs. As far as the mechanism of movements in the node is concerned, he considers it as a special case of "tissue tension," which he discovered.

In 1866, Bert [4] gave a very hypothetic explanation about the origin of the sleep movements. He assumed that they are formed under the influence of substances determined by light, which then were supposed to be converted in the evening into an endosmotic form. Thereby a change is supposed to occur in turgor which results in leaf drooping. /4

Batalin [2] (1873) found that the movement of petals occurs through uneven growth. However, he thought erroneously that these explanations should also be valid for the movement which is caused by the nodes.

The first thorough, fundamental study of nyctinasty appeared in 1875, written by Pfeffer [28]. I will briefly report the most outstanding facts of this study. Through his investigations in 1907 with the self-recording method, he comes to the same conclusions. There I shall further pursue the subject. By reduction of illumination, an increase follows; by increase of illumination, a lowering in turgor pressure follows in the nodes in both antagonistic halves simultaneously and in a similar direction (that is, either in both an increase

or in both a reduction "in the same direction"), but with different speeds. Pfeffer had thus a different opinion about it than Sachs had in 1857.

Alternating illumination exercises a stimulus upon the leaf whereby it is going to droop, and then rise again after some time. When constant light or constant darkness follow, this stimulus will remain in action for some time in the form of steadily diminishing aftereffects.

However, every day more new stimulus is added and by the interaction of aftereffects and new stimuli, daily movements will occur.

In constant light or darkness, the movements become less, finally to cease entirely. The daily alternation of light and darkness is therefore, according to Pfeffer, not to be considered as a periodicity of an ability which is fixed entirely by heredity, but it may be considered as the direct cause of periodic movements.

The autonomous movements which are continuously carried out are entirely independent of the daily periodic movements. Thus they constitute the third type of movement possibility, which Pfeffer distinguished.

Charles Darwin [12] was also interested in sleep movements of different plants. He attempted to find out its use for the plants, and then came to the conclusion that the vertical position during the night is supposed to protect the leaves against too great radiation. He demonstrated by tests that when the leaves were forced to remain in a horizontal position at night, they had much more to suffer from this radiation.

Through the investigations of Pfeffer in 1875, Darwin became convinced of the fact that the alternation of day and night is the cause of sleep movements. Yet he thinks that this alternation occurs perhaps more periodically; for, in the morning hours, when it is still dark, the leaves again go upwards, when the light is still unable to make its influence felt. Moreover, some leaves are still making movements in the dark for a whole day (!). He is unable to agree with Pfeffer's "aftereffects." Thus, he finds for the leaf movements: "We may conclude that the periodicity of their movements is inherited to a certain extent."

Darwin could establish for a very large number of leaves that the leaves carry out circumnutations. Sometimes the tip of the leaf describes one ellipse per day, sometimes several. Whenever these movements become more regular in



a number of plants by the alternation of day and night, nyctinastic movements arise.

He also mentions a very extensive list of plants whose primary leaves make sleep movements (53 plants), while for 86 species he states that the leaves show nyctinastic movements.

Darwin experimented with almost all these plants, so that he himself was able to determine the most prominent characteristics for every species. Although nowadays we contemplate geotropism, phototropism, nyctinasty, and other movements in plants, nothing more than modified circumnutation as far as Darwin is concerned was known; we are still greatly amazed about the enormous work which this exceptional investigator of nature produced with respect to this problem, too.

In 1891, A. Fischer [15] published his tests on the effect of gravitation on the movement of leaves. Whenever he made the leaves turn around the horizontal axis of a clinostat, the sleep movements apparently decreased in intensity next morning, and after a few days they ceased entirely. After the leaves were brought back into their normal position, after 24 hours the movements were again completely equal to those seen at the start of the test. /6

If *Phaseolus* plants are set down upside down, the sleep movements are also reversed. On the other hand, when he placed *Cassia* inversely, sleep movements continued normally.

Therefore he distinguishes: A) Geonictotropic leaves. The sleep movements reverse when the plant is placed inversely. *Phaseolus spec.*, *Lupinus albus*, *Gossypium*, and others, belong to this category.

B) Autonyctotropic plants. The sleep movements are normal when reversed. To this group belong, among others, *Trifolium pratense*, *Acacia spec.*, *Mimosa sp.*, *Cassia sp.*, *Oxalis sp.*, and *Biophytum sp.*

Thus, according to Fischer, sleep movements are caused not only by alternation of light and darkness, but also by unilaterally acting gravitation. This geotropic induction is not supposed to be necessary every day to bring about the nyctinastic movements. For if they are eliminated by letting the plants rotate on a horizontal axis of the clinostat, the movements cease only for a few days, although, beginning on the first day, they are decreased in extent.

Thus Fischer does not seem to accept a periodicity fixed by heredity.

After Pfeffer, different investigators were occupied with the question of a nyctinasty. Among them were those who concerned themselves exclusively with the opening and closing of flowers, which, as was already mentioned, is caused by an uneven growth of the two sides of flower leaves. Because the results of this are only indirectly connected with leaf movement, I shall discuss it only briefly. For example, Oltmanns [26] distinguished a "Frühschliesser" [early-closer] in 1895, which is a flower that opens in the morning between 6 and 7 o'clock, and on brighter days closes between 9 and 10 o'clock. When illumination is moderate, this type remains open the longest, while in very strong light or in very little light, it closes sooner. An example of this is *Tragopogon brevirostre*. /7

Opposite to this are the "Spätschliesser" [late-closers], which open in the morning between 7 and 9 o'clock, and close between 1 and 4 o'clock; *Bellis perennis* is an example. In general, he found that illumination helps opening; while darkness promotes closure, albeit this occurs more readily in the afternoon than during the morning. If flowers are placed in darkness during the day, they open earlier than usual the next morning. Oltmanns concludes from this that darkness can also promote opening. However, if the plants are illuminated at night with two Argand lamps, they will open later. Thus, Oltmanns shows that light promotes closure.

It is true for all flowers that they open in constant light, and remain open. In constant darkness, they close and remain closed.

We have seen that according to Darwin [12], the benefit of sleep movements is supposed to lie in the fact that the movements should protect the leaves against strong cooling in the warmer regions of the earth and, in particular, against frost in the spring at higher latitudes. Stahl [43] (1897), however, looks for another explanation. According to him, the leaf will be covered with dew at night because of the vertical position, whereby evaporation, and the supply of inorganic substances, is supposed to be blocked. Indeed, he could establish in numerous tests that horizontal surfaces will be bedewed more heavily and earlier than vertical ones. He also noticed that leaves which mostly evaporate on the underside will bend downwards in the evening. The leaves which evaporate most intensively on the upper side will move upwards,

so that the side evaporating most is always protected. *Oxalis*, *Robinia*, etc., belong to the first group. *Medicago sativa*, *Trifolium repens*, and others are of the second category. This can be different even within a genus; thus *Impatiens parviflora* belongs to the first, while *I. glanduligera* belongs to the second group.

Thereafter, in 1897, we find a detailed article by Jost [20] on the periodic movements of the leaves of *Mimosa pudica*. His testing arrangement was such /8 that he kept the topmost part of the plants in a large light-proof box (fastened with cork and wax) and then made tests at different temperatures and illuminations. In this manner, he obtained thoroughly etiolated leaves. The lowest leaves, which were not kept in the box, could continue to assimilate. He does not mention what arrangement was made for moisture and air refreshment. When free green leaves were kept in the dark for a whole morning, he observed that they open much later than the others, but he sees no difference in movement in the leaves in the box. Jost thus argues that there is no stimulus-conducting place from one leaf to the other.

He found in his temperature tests that rapid warming from 20° to 38°C was followed by a closure of leaves. They remained closed at noon; however, in the morning hours they open even at the higher temperature. He ascribes the cause of periodic movements to the every-day change in temperature; because, when he kept the temperature artificially high at night, and lower during daylight, the leaves assumed a diurnal position at night, and a sleep position in the morning.

Generally, warming acts as an increase in illumination. The leaves open; and vice versa, the leaves close at cooling and darkening. Yet, rapid and high heating has another sequela from that described above. I shall quote here word for word Jost's complicated and very twisted hypothesis for the explanation of nyctinasty: "After the leaves opened during the early morning hours, they transfer to the night position as a result of a temperature rise. If this temperature increase occurs not too early, then the closure which it will cause will be a lasting one. However, if it occurs early or rapidly, the tendency of the leaf to keep the leaflets in a diurnal position can be overcome for some time. However, an opening movement occurs soon after this first closure, but one which will never put the leaflets into positive diurnal position. When

the temperature continues to rise, the leaflets remain closed, or close anew, /9  
respectively. And the cooling that starts toward the evening will not initially  
change anything. Only when cooling lasts longer, and has a greater intensity,  
a transition into the diurnal position will occur next morning."

We can see that it is not simple. For that matter, with the whole method  
of experimentation something and another is certainly to be found faulty.  
Illumination and warming in a considerably small box, in case of such highly  
sensitive plants as *Mimosa pudica*, must bring results which unequivocally  
could be subject to doubt.

The following year we find a publication of Jost [21] (1898) on the  
nyctinastic movements of petals. Pfeffer and Oltmanns found that flowers  
open to a certain limit when the temperature rises, from which they will close  
again although the temperature increases further. Jost checked this finding  
and also finds that a reverse movement of the petals follows after every flower  
opening caused by rising temperature, when this higher temperature remains  
constant. This reverse movement may last for hours, without, nevertheless,  
leading to a complete closure. When a temperature increase occurs again, during  
this reverse movement, the leaflets start to open again, etc. Thus we can  
gradually bring about a continuous opening. At a higher temperature, differences  
must also be larger.

Jost also carried out a few tests on the mechanism in the stem nodes of  
*Mimosa*. We have seen that Pfeffer assumed that the upper and lower side of  
stem nodes react alike. Jost finds, however, that when he cuts away one half  
of it, i.e., the upper or the lower half, the leaf movement remains unchanged.  
But this is only possible when both sides of the stem node (geniculum) respond  
unequally to light and darkness.

Many other investigators have tried to explain the mechanism of leaf  
movements. Thus, in 1898 Schwendener [38] comes to the conclusion that the /10  
two halves of the stem node respond in an opposite direction.

Pantanelli [27] (1901) however, is of the opinion that the two node halves  
show a similar response, for when only the upper half is present, a movement  
occurs which is similar to the one in the plant as a whole. If only the lower  
half is present, then the reverse movement occurs. Pfeffer found that turgor  
movements are supposed to run in the same direction, but with uneven speed.

As a result, the leaf should return by itself to the daylight position in the morning (when the pressure becomes again equal in the two halves of the stem node). Pantanelli cannot find this return, so he rejects this part of Pfeffer's theory.

In 1904, Wiedersheim [55] instituted extensive research into this question. Just as Pfeffer, he finds that as a result of darkening, turgor increases at the upper side, followed by an increase in the pressure on the lower side, whereby the leaf returns again to its starting point.

The difference between Pfeffer, on the one side, and Jost and Schwendener on the other, is a difference in the cut-away according to Wiedersheim. When he cuts away the node up to half of the vascular bundle, he obtains the result of Pfeffer; with a less deep cutting, he obtains the same findings as Jost and Schwendener. In *Impatiens*, he keeps the leaves horizontal with a weight, and then looks for what kind of effect an alternation of light and darkness has upon the growth of the upper and lower side of the leaf. The upper side starts to grow quicker first, then the lower side follows, with the result that the midzone also experiences an acceleration in growth. Thus, he was able to determine the normal sleep movements and found that two hours after the start of darkness, growth acceleration of the upper side stopped; meanwhile, three to four hours later the lower side began to grow more rapidly, with the result that in morning around five o'clock the leaves were again back to their starting point.

Then he made the same thermonastic tests with tulips and found again: first growth acceleration of the upper side, with the lower side growing slowly. Following was the reverse, delayed growth on the upper, accelerated growth on the lower side (the temperature rises from 5° to 29°C), and in the midzone, double acceleration.

/11

With the aid of graphics, he manages to show these results clearly.

In 1904, a very remarkable book by R. Semon [39] was published, entitled The Mneme as Conserving Principle in the Exchange of the Organic Process. He starts with the following theory: when an individual encounters one or another stimulus, it will always affect a change. He calls this the engraphic operation of the stimulus, and the supposed change on the organism is called an "engram."

Then, when the same stimulus affects this individual once more, with one engram already present, this second stimulus may be less strong to cause a similar response. These engrams also can pass on to descendants. This explains what until now we had called "hereditary predestination." It is also very important that the phenomena take place in the usual manner whenever the conditions are not entirely and fully satisfied. This, then, is supposed to be the great difference between true mnemic and non-mnemic phenomena.

According to Semon, all organisms have the capacity to absorb engrams. He calls this charactersitic "mneme."

He then attempts to explain all possible physiological problems with his theory.

In 1905 [40], he used his mneme theory as an explanation of nyctinasty. He tried to revert or change the daily cycle of Acacia sprouts through artificial illumination, for, he says, when this is done with success, this is a proof for the engraphic operation of stimuli upon plants. However, he did not succeed in inducing a period of 24 hours with sprouts of Acacia lophanta, or with sprouts of Mimosa pudica. He alternately provided 24 hours of light, 24 hours of darkness. The light intensity was 25 candles. However, he explained this negative result by assuming that the engrams of the daily movement offer too much resistance, and that they have a long duration of effect. /12

Then he started to grow plants from seed under alternating illumination conditions, but not according to the normal daily period. As soon as these plants had developed to the stage where they carried out distinct movements, they were brought into constant light or darkness. In all these cases, Semon stated that leaves had definite 12 hour periods, although they had never been under similar conditions.

Even during intermittent illumination of 6 or 24 hours, the 12 hour period was well visible.

He states that when leaves have lost their motility by constant conditions, they became insensitive to stimuli at the same time. This is in contrast to the findings of Pfeffer, who found that when "light or darkness rigidity" occurred by darkening or illumination, respectively, a powerful response is carried out.

Semon assumes that periodic movement is hereditary, whereas according to Pfeffer, the ability to respond to alternating illumination with a periodic movement is hereditary.

The following will give us an idea about Semon's method of experimentation. The tests were made in a dark box, where the temperature could not be kept constant and humidity was not taken into consideration, while lighting was constant with a 10-candle lamp!! How often per day, and how he determined the position of the leaf, especially in constant darkness, is not indicated.

For the sake of clarity, I shall discuss the articles of Pfeffer and Semon one after the other. The proceedings of other investigators will be discussed thereafter.

A far-reaching, important work of Pfeffer [29] on the nyctinastic movements was published in 1907. This ushered in an entirely new period in investigations, for now Pfeffer used a method whereby all movements of the organ were recorded by means of an indicator on treated paper in a kymograph. An extensive discussion of this method can be found on page 34 of this work. During the discussion of my own investigations I shall have the opportunity to repeatedly point out additional details from the work of Pfeffer. Let it be sufficient here to give the most important results.

/13

The sleep movements are responses which are caused by the daily alternation of light and darkness (or of temperature, for flowers).

They cease gradually at constant light or constant darkness. However, the plants remain able to react, as appeared in the tests. The same is true whenever plants are grown entirely in constant light: they show no trace of periodic movements, but they certainly are able to react.

The sleep movements of leaves of *Phaseolus*, for instance, are photonastic responses, i.e., caused by the alternation of light. Consequently, it is possible to shift the direction of movement quickly by 12 hours, so that leaves come into daylight position in the evening, and into night position in the morning.

(Pfeffer does not mention the employed light intensity; he only indicates that he worked with 4 tantalum lamps.)

The leaflets of *albizzia* and *mimosa* respond immediately, both to a reduction

and to an increase in illumination. The drooping of these leaflets in the evening should therefore be a direct sequela of the arriving darkness.

According to Pfeffer, *Phaseolus* reacts exclusively to illumination, with the response beginning a few hours after the start of stimulus. Accordingly, the light in the morning should be the cause that the leaves assume the sleep position in the evening.

We must not imagine that one or another sudden disturbance is supposed to occur in the plant by the transition of light into darkness, or vice versa, but that gradually a new equilibrium is obtained, which matches the changing light or temperature conditions.

Yet, Pfeffer must draw the conclusion from his tests that in leaves of *Phaseolus* and many others, a tendency is present for assuming an approximately 12:12 rhythm of movement. Even when leaves have assumed an 18-hour period as /14 a result of artificial illumination (18 hours of light and 18 hours of darkness), and thereafter are subject to constant conditions, the residual swingings are supposed to take up once more the normal rhythm.

That the autonomous movements are independent of the sleep movements, is evident according to Pfeffer from the fact that the latter cease in constant light or darkness, while the former continue to go on, often with a greater amplitude. The autonomous movements are due to an inherently present motility, but they are almost completely covered up by the sleep movements, so that they become apparent often only after the latter have disappeared under constant conditions.

Although in this document Pfeffer strongly disputes Semon's concept that nyctinasty is a property fixed by heredity, we still have the impression he doubts it in some way. This shows, for instance, in his above-mentioned remark about the normal residual swingings of differently illuminated plants.

Of course, Semon did not leave this document of Pfeffer without an answer, the more so because Pfeffer made a rather sharp criticism about his less exact method of experimentation.

Semon, as one would expect, did not attempt to strengthen his arguments by more and more reliable tests, but declared in a detailed article in 1908 [41] that the tests of Pfeffer are very nicely in corroboration with his (i.e.,



Semon's) theories. Although he allows that Pfeffer uses a stronger light source and a better method, the disadvantage of the "strong" light (25 candles) is that by such a powerful intervention the inherently present periodic motility is directly suppressed, while by his weak light it can manifest itself.

The self-recording method is, according to Semon, unsuitable for the sprouts of Acacia and Mimosa, which he used, because the leaflets are particularly delicate; he would sooner use the angle reading method, regardless of how tiring it is.

He did not want to eliminate the heat function of the light rays, because /15  
"this way the conditions were closer to natural conditions."

According to Semon, the greatest difference between his and Pfeffer's experiments is that Pfeffer, by using Acacia and Mimosa, always worked with large plants, which had already been exposed for some time to the alternation of light and darkness, while he used plants which were grown from seed under abnormal conditions. Pfeffer suppressed first the motility with constant light, and then, with much stronger light, induced another period which did not end in a normal rhythm. Thus, says Semon, if Pfeffer had used the same test conditions as he, then Pfeffer should have obtained the same result. This seems to be the case because in *Phaseolus* Pfeffer found indeed an "indication" of a tendency to pass into a normal rhythm (I have already discussed this).

Then, numerous insignificant remarks on Pfeffer's works follow, and he finishes with the assurance that Pfeffer "is substantially in agreement with Semon's theory, so that now there is a satisfactory agreement between Pfeffer and me. The differences are of a quantitative nature only."

However, there is one area with respect to which Semon admits that he actually has an entirely different opinion than Pfeffer. This concerns the aftereffects. According to Pfeffer, we must consider them simply as residual swinging, as the swinging of a clock, which received a push. Here he uses the expression "unstimulated state." Semon, on the other hand, shows that sleep movements are carried out during infinite generations in a rhythm which recurs daily, so that they have left behind an unmistakable engram, which, of course, is expressed also in the aftereffects.

Whenever the light or darkness are constant, according to Semon we cannot

speak of a stimulus-less state; this can be done only when there is actually no more stimulus to act upon the organism. When we keep the plants in constant light, stimulus is applied every 12 hours. The aftereffects of these constantly equal stimuli are supposed to counteract the original periodic aftereffects of daily movements, so that finally a standstill occurs. Thus, there is /16  
no question of fading out, but of a suppression of movements.

Because most plants react to darkening to some extent, we can adapt this theory also to the case of constant darkness.

It is worthy of note that Pfeffer, as well as Semon, report that constant light seems to be more harmful for plants than alternating illumination. I mention this fact here only because during the processing of my tests we will see that I have come to the same conclusion.

It is obvious that Pfeffer did not let this article pass by without remarks. In the same year (1908) [30], we find his refutation in the Biol. Centr. Blatt.

He explains first very explicitly which viewpoint he holds in this question: "From the experiences of the dependence of the environment (i.e., alternation of illumination and temperature) it had to be, and must be, concluded that the sleep movements, and consequently the residual swingings, are not based upon motor activity transmitted by heredity, as is the case in autonomous movements, but that they originate through the rhythmic repetition (photonastic and thermonastic) of stimulus impulses. However, in order for the external influence to have the result mentioned, the object must be equipped with the appropriate qualities, i.e., with a corresponding ability of response (including the ability of movement), which the object possesses as inherent, a characteristic transmitted by heredity."

According to Pfeffer, there are many additional, specific features to be observed in different plants. He also lists here the deviations found by Semon. Pfeffer thinks he is able to conclude from Semon's work that he, too, considers the daily movements as photonastic responses, so that there should be no substantial difference between their concepts!

We have already mentioned that Semon does not consider the movements in constant light or darkness as residual swingings of a pendulum, but that, according

to him, they are supposed to be the mutual effect of new stimuli and so-called /17 residual swingings. Contrary to this, Pfeffer justly quotes that in that case we might speak no more of residual swingings in the case of a pendulum; after all, even in a pendulum, the movement is continuously counteracted by resistance, i.e., air resistance. If this were not so, the pendulum would continue an unlimited movement. Under constant conditions, there is no occasion to movement, and thus the organ remains stable in a definite position.

Again, Pfeffer mentions his distrust of Semon's results, because of his not keeping the temperature constant, too little illumination and incomplete angular readings.

Whenever we compare these two investigators, we always find the great accuracy and reliability of Pfeffer's experimentation. First, he takes tests, and then tries to explain the results either by setting up a new hypothesis, or by bringing them in agreement with a former work hypothesis. Semon, on the other hand, sets up first his mneme theory, and then tries to incorporate the results of too few tests.

I was unable to find any further article by Semon on this subject.

In 1909, we come upon a very remarkable document of Lepeschkin [23]. First of all, he has also investigated how the two node halves react. Just as Pfeffer, he finds that after darkening these react in the same manner, but at uneven speed, provided that the node is cut through to the half of the vascular bundle. If he cuts less deeply, he obtains the same results as Schwendener, uneven response in the two nodal halves.

He is able to state that the mechanical properties, as well as the bending stability, never change in the nodes. Thus, the movement should be explained by a change in turgor pressure.

By light the protoplasmic permeability should decrease, and in connection therewith the osmotic pressure of the cells.

He tries to explain this hypothesis in three ways:

A) He placed 400 *Phaseolus* leaves in the dark, and 400 in the light. /18 After a few hours, the nodes are cut up and extracted. It appears that the quantity of substances exosmosed from one gram of nodes is about one and a half times as large in light as in darkness.

B) The nodes of *Phaseolus* and *Mimosa* were cut into thin slices with a microtome. One half of it is placed in darkness, the other half in light. After some time, the nitrous content of a number of slices of each portion is determined by plasmolysis. It is clearly shown that in light the permeability might have been larger.

C) He determined the isotonic coefficients in light from node slices of *Phaseolus* and *Mimosa*, and thereafter they remained for a certain time in darkness. He obtained the same results.

He then investigated whether this remarkable property of the plasma also occurs in other plant parts. Indeed, he found that the permeability of the plasma of the *Tradescantia discolor* epidermic cells is 1.5 times larger in light than it is in darkness. In *Spirogyra*, it is 1.8 times larger. His tests showed that the permeability of cells of the upper half of nodes of *Phaseolus* is always greater than the lower half. From this he concludes that "after darkening, leaf movement occurs due to an uneven plasma permeability for dissolved substances in the cells of the upper and lower halves of the node. In the half wherein permeability is greater, a greater increase in turgor pressure will be produced by darkening which moves the leaf toward the side of the half of lesser permeability." That the leaf thereafter returns to its original position is, according to Lepeschkin, not a sequela of light decrease that occurred in the place, as Pfeffer has assumed it, but must be considered a sort of after-effect, in the sense of the aftereffects in constant light or darkness.

Even though the results of L. seem remarkable, we should not lose sight of the fact that all methods used are very unreliable because of wound stimuli, fluctuations of temperature and illumination, while sometimes very complicated /19 formulas were used.

The following year (1910) brings us a publication of Trondle [53] on the same subject. From his tests with *Buxus* and *Tilia*, he finds that permeability decreases in light, and increases in darkness. This belongs, he says, to the living protoplasma; it is not a simple photochemical process, but a "stimulus process." By determination he sees that in nature the permeability in the plants mentioned increases from December to July, and then decreases again.

With respect to this, criticism was heard from various sides, particularly concerning the methods used by him. Fitting [16], for example, said in 1915: "The observation, which should be pursued by further experiments, that, as opposed to the findings of Trodle and Lepeschkin, light did not show any influence on permeability is very remarkable. On this occasion I would be remiss in not pointing out that, after extensive research in the plasmolytic method and in the questions related to it, I regard these investigations very skeptically, particularly because the method of determining the permeability coefficient in the way it is used here is misleading, because it does not sufficiently take into consideration well-known facts of physical chemistry."

However, in 1922 Brauner [8] was of the opinion that he found in the coleoptiles of oats that light influences the permeability of plasma. He determined the permeability with the improved Fitting method and found that permeability of coleoptiles which were in light was 75% less than in those which were in darkness. It is unfortunate that Brauner's tests leave many things to be desired, so that we must consider the results with great caution.

For that matter, the same was found for the nodes of *Mimosa pudica* by Blackman [6] and Paine in 1918. They kept the cut off nodes in a small glass /20 cellule with very little water. It was found that in almost all cases such a node was still able to react normally. Two platinum electrodes were inserted in the liquid of the cellule. The tests were made at constant temperature and humidity. The changes in the osmotic pressure could then be determined, by measurement of the conductive capacity of water in the cell. Of course, with an exosmosis from the node this pressure increased. The protoplasmic permeability in the node increases by illumination.

In 1910, R. Stoppel [45] published her results on the influence of light upon the opening and closing of a number of flowers. With a glass capillary to be fastened on the petals, the angular measurements can be regulated. The flowers of *Calendula* open in constant darkness, and make movements which proceed in a daily rhythm, and therefore must be autonomous. On the other hand, the buds open very slowly in constant light, and then they make no movements. By applying another period of light and darkness, the opening and closing of flowers also changes. Thus we can introduce any arbitrary period, and always make the flowers open at illumination and close in darkness. This is true, however, only as long as the period does not drop below 6 hours of light : 6 hours of

darkness. For instance, in the case of 2:2, there are no aitionastic reactions to be seen any longer. Then, the autonomous movement again appears in the normal daily rhythm. If the tempo of the light and dark period is slower than normal, then the flower adjusts itself again, but in the sense that the opening occurs during darkness and closing in the light period.

Furthermore, she finds that in the case of illumination, the flowers open, just to close again a few hours later. Therefore, Stoppel ascribes a double effect to light: a "transition reaction" and a "sequential reaction."

Whenever she grew plants from seed entirely under abnormal conditions, flowers in constant darkness still carried out autonomous movements in a daylight tempo, so that this must be a hereditary characteristic. She also finds /21 that the transition reaction is strongly dependent upon the disposition of the light on flowers: we saw that no movements are carried out in constant light, but after brief periods of darkness, the disposition is changed so that in case of relighting, a reaction follows quickly. Thus, after a shorter light period the following dark period must last longer in order to get the same result. Because the reaction is also dependent upon the phase wherein the movement of the petals happens to be (i.e., whether they are to open or to close), different responses can be obtained by one and the same change in the illumination at different times, and with different preparation of plants.

With *Bellis perennis*, she gets somewhat different results. The buds do not open in constant darkness; in constant intensive light for a few days they make periodic movements in an approximate daylight rhythm, only with ever-decreasing amplitude. Furthermore, *Bellis* resembles *Balendula* in reactions. The autonomous movements appear in a less obvious manner, so that they have also less influence upon the transition reaction which is likewise present here.

Stoppel [46] and Kniep made further investigations with *Bellis perennis* in 1911. They also found a closure response in constant light, and a delay of opening, whereas the tendency of flowers before darkening is very much dependent on the duration of the preceding light period. If a short dark period follows a long time of constant light, a strong opening movement will follow as a response during the following illumination. Of course, here

it is of great importance whether the flowers are then in the phase of opening or closing. For instance, if the dark period falls upon the moment when the flowers are just about to close, the phase of rhythm and transition reaction of dark upon light are operating in an opposite direction. If it comes at the moment when the flowers are just about to open, they operate in the same direction.

When closed buds are exposed to constant light, they do not open. If they are open to some extent and a dark period of at least three hours went beforehand, then here again the already mentioned light transition reaction on the opening of the flowers can be noticed. If the light continues, the flowers will again close. If they are then exposed to darkness, they completely close, open again and, when in constant darkness, carry out rhythmic movements. /22

The daily opening and closing is thus explained: because of darkness, they close in the evening. When constant darkness continues, they should open the next evening, but because there is light in the morning, the transition reaction to light takes place, whereby they will open to the maximum about 12 o'clock noon. By long-lasting light, we then get the "sequential reaction," which makes flowers close, aided by the again oncoming darkness.

In 1911, Pfeffer [31] initiated an investigation into the effect of a mechanical arrest of sleep movements. A stalk of grass fastened to the midrib of a leaf of *Phaseolus* rested on another stalk which, with the aid of a steel wire, was hinged at one side with the other end attached to the tracing wire of the indicator which records on the kymograph. This generally stops the sleep movements, and this effect is then recorded by the indicator. In addition, a small weight can be suspended under this leaf, or the small weight can be run over a small pulley, and exert a pull on the leaf.

In all these cases, he found the same reaction as in a normal leaf. The daily movements occur as usual, even though with a smaller amplitude. A reversal of the movements is possible by illumination at night, and darkening in the daytime. In constant light the sleep movements stop, and autonomous swings appear. It follows that the processes which induce a movement can occur even when the node is artificially hindered in bending. They are not dependent on a reaction which takes place after the node bent initially. In case of a stalk of *Momosa pudica* and M. Speggazini, a counterreaction occurs when pull is directed downward or upward by a weight. When the stalk is /23

pulled upwards or downwards by this weight, a movement in the opposite direction follows, which brings the leaf back to its original position. The same takes place whenever the leaf is taken out of the balanced position by the removal of the small weight; then also a counterreaction follows.

If a *Phaseolus* leaf fixed in its deepest nocturnal position is again released the next morning, it completely assumes its daylight position. And vice versa, a leaf fixed in daytime position, which was released in the evening, reaches its night position. It follows from this that, even when the bending of the node is mechanically obstructed, the same tension intensity develops that is normally formed in a bending node. Thus, Pfeffer could establish that in the powerful nodes of *Phaseolus vitellinus* the corresponding static moment amounted to 160-545 grams.

Very remarkable is his discovery that *Phaseolus* leaves continue to make normal periodic movements in constant light, when the node is surrounded by black cotton-wool. Without these cotton paddings, short, periodic swings were made. In 1915, Pfeffer [32] returns to this in more detail; I shall therefore pursue it further. With a node exposed to darkness, the period of sleep movements can be still reversed. Thus the blade exerts great influence on the movements of the node.

After Stoppel found in 1910 that the flowers of *Calendula* carry out movements in constant light in a diurnal rhythm, we find in 1912 [47] a preliminary report which stated that she succeeded in growing *Phaseolus* sprouts in constant darkness, until the primary leaves were well developed. These etiolated leaves carry out movements in constant darkness which correspond to the usual sleep movements of green plants. This should indicate that sleep movements also have an origin fixed by heredity.

In 1915, Pfeffer published his last great investigations on nyctinasty. Based on his own investigations in 1911 and the publications of Stoppel, he began to doubt whether sleep movements were merely photonastic responses. In this research he included the following plants: tulip blooms, leaflets of *Albizia lophanta*, leaves of *Phaseolus vulgaris* and *Flemingia congesta*, and the main stalk of *Mimosa spegazzini*. As a result, he made a sharp distinction between two causes which were considered for the origin of sleep movements:

- a) An ability to carry out autonomous movements in a normal, i.e., diurnal,

/24



rhythm. This we find in *Phaseolus* leaves, which certainly continue their movements in constant light, provided that the node is made dark, and which also under certain circumstances do not show any rigidity ("darkness rigidity") in constant darkness. The *Calendula* flowers which continue to regularly open and close in the darkness, also belong to this category.

b) An ability to carry out exclusively photonastic responses, such as the blooms of tulips, which carry out some movements both in constant light and in constant darkness.

When the *Albizzia* and *Flemingia* leaves do not make any further movement due to constant conditions, they can be restimulated by a periodical illumination, but then the size of the movement amplitude very much depends upon the size of the stimulus. This indicates a photonastic character of movements. The reversal of sleep movements by illumination at night and darkening in daytime is very easy in both species. It is also peculiar that this photonastic stimulation has the same result whenever the node is surrounded by a black cotton pad, and the blade is illuminated, or the blade is kept dark, and the node is illuminated. According to Pfeffer this is a stimulus conduction. Meanwhile, this does not seem to have taken place throughout the entire plant; for when the two leaves of a plant are kept under different conditions, each carries out a movement which fits the conditions.

All leaves, whether they belong to type a or type b, have additionally characteristic small swings which are entirely autonomous; for under completely /25 constant conditions they are continuous without weakening, and often become more conspicuous.

This document of Pfeffer shows that he, who so violently attacked Semon in 1908, because he called the sleep movements autonomous, now was himself forced to accept this explanation to a great extent.

As we have seen, R. Stoppel was also initially of the opinion that nyctinasty should be an autonomous phenomenon. In 1916 [48] she refers to it in an article which gives an entirely new and very original explanation for this remarkable phenomenon. She carried out experiments in the dark with etiolated plants, and also with normal green plants in an experimental box.

The arrangement and recording was the same as with Pfeffer. It is unfortunate

that the indicator gradations used for the kymograph recordings were not always the same, so that the curves which she published cannot actually be compared with each other. Another great drawback, which I have against this document in general, is that Stoppel used her plants so long that they died. We read repeatedly under her curves that the leaf rolled up and was ruined. However, a completely healthy leaf does not die so suddenly, so that in part, she actually recorded the movement of not entirely normal plants. Externally they may still have a normal aspect, while internally the dying processes may have already begun.

The effect of temperature upon movements appears to be slight, at least fluctuations of  $2^{\circ}$ - $3^{\circ}$  remain without effect. Greater differences,  $7^{\circ}$ , bring on irregular movements. With the help of a clinostat and centrifugal tests, Stoppel comes to the conclusion that unilaterally acting gravitation is indispensable for the development of sleep movements, but that it is not the direct cause.

Now, it was known to her that electric conductivity of air also shows periodic diurnal oscillations, the peak of which is in the morning about 4 o'clock.

/26

Still another factor could be the potential drop. She states: "In normal times, the atmosphere is positively charged with respect to the earth. The average potential difference is 100 V per meter. If we imagine therefore a 20 m high tree, then between the top and the root of the tree there is an average potential difference of 2000 V. Potential differences are smallest at night, and greatest during the day." In closed rooms, however, there are no potential differences, so that only the conductivity comes into consideration as a possible cause of nyctinasty. She tries to verify this hypothesis with many tests.

She repeatedly observed that the simple touch of a bean plant will start a temporary disturbance in the picture of movement. This happens because the plant has a certain charge which is "discharged" by touch. This charge enters the plant from the ground through the roots. When a plant is isolated from the ground, this might not be without influence upon the movements. She therefore placed an (etiolated) plant on a thick glass plate, and this again on a piece of cork. Indeed, the results were somewhat lower, however, when we look at the curves (for instance 22 and 23 on page 643), it seems that the decrease

is not significant. It is conceivable that we are dealing here with an accidental deviation. Then, a plant was set up in isolation in a cage of fine-meshed iron wire, thus the atmospheric electricity now cannot reach these leaves. The movements become less, and finally cease. When then after a few days the plants are again placed on a table, the sleep movements return immediately.

When such a plant in a cage did not make any more movements, the cage was charged with 1 V, and the movements of leaves returned. If the cage is connected with the city network of +220 V, then the sleep movements continued without disturbance, while they stopped after two days when the cage was charged with -220 V. In case of green plants, she obtained the same findings. The influence of isolation can also be well observed here: "The leaves looked sick, droopy, /27 and the leaf ribs bent like the ribs of an opened umbrella, even when the spread had been previously nicely extended."

In green plants a negative charge of the cage was unfavorable, and a positive charge favorable for leaf movement.

If, in fact, the conductive capacity of the atmosphere has an effect, a change of it should be noticeable in the movements. She tried to modify the conductive capacity of the air by placing ashes of a burnt-out gas mantle in the vicinity of the plants. Actually, disturbances did occur in the movements of leaves, which disappeared as soon as the ashes of the gas mantle were removed.

This proved to R. Stoppel that the conductive capacity of the atmosphere is the cause of nyctinasty. In the theoretical part of this article, she cited all possible examples from the literature to prove that electricity is of the greatest importance for plant life.

At the same time, she reported all phenomena from plant and animal physiology which show a diurnal rhythm, such as heartbeat and blood pressure in man, root pressure, respiration, mitosis, and so on, in plants. These all should then be explained by assuming that they are influenced by the periodicity in the conductivity of the air.

The greatest objection I have against this publication of Stoppel is that she first builds up her theory and then tries to prove it by tests. Also, the reproduced curves are such that it could easily be taken as negative proof for this theory. Often she made too few tests which are actually "striking."

Sometimes a test is successful only once, and yet it is taken to be a valid proof!

When Stoppel published this paper, she knew that conductive capacity of the outside atmosphere showed periodic oscillations. Whether these occurred also in closed rooms, as in the dark chamber where she carried out her experiments, was not known, she had reason to suspect it. In the following years she thoroughly pursued this question. In 1919 [49], she published her results on this subject. Because this is pure physical research, I will be satisfied with reporting the data that are most significant for us. Indeed, the conductivity of the atmosphere changes in closed rooms at constant temperature every day with a definite rhythm. Between 2 and 4 o'clock in the morning the dispersion is the greatest. Light causes a marked increase in this dispersion. During the light period the periodicity is less obvious, but the small oscillations are considerably stronger. /28

Stoppel once more devoted herself to the field of electrophysiology. She tried to bring other periodic phenomena into connection with it. Thus, in 1920 [50], we find her doing research on the influence of emanation upon respiration. She found that when the ion content and the air conductivity are increased by letting the air pass first along a small pipe with carnotite before it reached the chlorotic branches of *Aesculus hippocastanum*, the respiratory intensity of it considerably increased. With green plants (branches), she found a few exceptions, while young pot plants in 2/3 of the cases showed an increase, in 1/3 a decrease, in the respiration.

This is followed by an extensive theoretical part, with all kinds of electrophysiological theories and hypotheses, which I do not intend to consider here.

It is understandable that Stoppel's investigation on nyctinasty was rechecked. However, it did not happen until 1922, when it was done by Schweidler [37] and Sperlich.

It is unfortunate that their method of experimentation does not make very reliable impressions. For instance, they could not heat their experimentation room, so the temperature from April to July varied between 14° to 21°. Now, 14° is undoubtedly too low a temperature for these tests. This may perhaps

explain why they received such flat, less pronounced curves. Their kymograph was also very primitive. The paper had to be taken off every day, and replaced by a new paper, which always caused a few disturbances. They started with tracking the influence of a mesothorium preparation upon the leaf movement of *Phaseolus*. But neither by radiation, nor by increased conductive capacity of the air was any change observable. They were able to corroborate Stoppel's findings that in closed rooms there is a periodicity of the conductive capacity of the atmosphere, similar to that in the free field. The chief peak of this conductivity falls between 3 and 4 o'clock in the morning. Its daily courses and those of leaf movements do not run parallel, however, so that we cannot speak of a connection. For instance, they could not find any definite time at which the leaves were in their lowest position. In 158 days this lowest position was reached between midnight and 11 o'clock in the morning. Stoppel, on the other hand, found the lowest position in the morning between 3 and 4 o'clock. Schweidler and Sperlich then try to explain this difference from the findings of Stoppel by assuming that the more even are temperature and other external circumstances in the "critical period" of the plant (i.e., from the swelling of the seeds to the beginning of the stretching of the epicotyl), the more regular is the picture of leaf movements. If germination falls upon a dark, cold day, weaker specimens are supposed to develop. According to them, Stoppel should have, and she did so without knowing it, raised her plants under practically similar conditions during the critical period, in fact even until the beginning of the test. She herself placed the seeds in water always at the same hour of the day. Accordingly, the sleep movements of her plants were supposed to show very great similarity. Due to their small number of tests, the two authors still are willing to state "that the completely autonomous nature of the movement in question should not further amaze us with regard to the life spent all the time under the alternation of light and darkness."

A remarkable article is that of K. Suessenguth [51], which in 1922 tried to explain "simple physiological processes," such as nyctinasty, with different data of physiological and colloid chemistry. It is very tiresome to state the outstanding results from this work, since it is written entirely in a brief, concise manner, just as a digest. Moreover, not a single curve is reproduced, while protocols and method of investigation are also missing. This prevents

any checkup on what he reports.

For example, extremely complicated physical chemical processes are supposed to take place in the nodes which are of great influence upon turgor, and thereby again upon leaf movements. Then, he proceeds to demonstrate how all kinds of substances influence nyctinasty; for he supposes that they cause definite chemical reactions which thereby modify the movement of leaves.

Among the examined organic and inorganic substances are some which are without influence upon leaf movements, while others make the leaf assume a new position. The cause of this is that these substances would make plasma colloids take in water, and then withdraw water from them. Finally he states that there is also a possibility that nasties and other tropisms are based on physico-chemical functions.

Finally, we come to the last great work which appeared on nyctinasty, i.e., the work of Hans Cremer [11] in 1923. As we have seen, part of Stoppel's tests were already rechecked by Schweidler and Sperlich, even though with negative results. Now, Cremer reexamined a greater part. First he gives a survey of the times at which *Phaseolus* leaves reach their lowest position. This is between 2 and 5 o'clock in the morning, both in winter and summer. We find here a great contradiction to Schweidler and Sperlich, who could not state any definite time.

Setting his plants entirely isolated on thick glass plates had not the slightest effect upon leaf movements, as opposed to what Stoppel thought to have found. Neither could he find a connection between climatological deviations and irregular leaf movements. Tests with electric fields in a charged cage, or with increased ionization, seemed to him not to have any effect on nyctinasty. The curve of the conductivity of the air does not run parallel with the curve 31 of leaf movements of green and etiolated plants. He directs attention to the fact that one often finds plants which, regardless of their being under entirely normal conditions, describe a deviating, abnormal curve. The cause of this cannot be determined. He used this as an explanation of the fact that Stoppel obtained different results, which deviate from those which he, and also Schweidler and Sperlich, found: they used such abnormal plants accidentally.

He could establish with many tests that emanation is not entirely without

effect upon the image of movement of the leaves. This emanation preferably occurs near charged wires. Now, the natural emanation content of the atmosphere in Basel, where Stoppel experimented, is greater than in Wurzburg where Cremer made his tests. Therefore, a greater emanation deposit should have been present on the charged cage of Stoppel than on Cremer's cages. This is supposed to satisfactorily explain the difference between Stoppel and Cremer.

We have seen that Schweidler and Sperlich explained the difference between their and Stoppel's tests by assuming that Stoppel treated her plants always the same before they were used in a test. Since this was not confirmed, however, by experiments, Cremer reared *Phaseolus* plants in the most divergent manner. Yet, all these plants yielded movement curves which very much resemble each other, so that the supposition of the two mentioned authors is thereby refuted, something which will not greatly surprise anyone.

The tests which Cremer carried out in an unused shaft of a salt mine are worthy of mention. The space where he experimented was entirely surrounded by thick salt layers, so that the temperature and humidity fluctuated very little. Here he raised plants entirely from seed, and then he makes the remarkable discovery that they do not carry out any daily sleep movements any more, although they were entirely healthy and able to respond; for when he raised the temperature 9° higher, a slow drooping of the leaves followed. If the temperature is then again brought back to the original one, after a while the leaves again reached their first position of balance. Certainly, these etiolated leaves make small swings. If such plants are then brought near the ground surface, and if they are set up under otherwise entirely the same conditions in a dark chamber, the leaves again start gradually to carry out normal daily movements. Tests with ionization, isolation, and with charged cages were, in Cremer's experiments, too, without any influence on sleep movements. He found, just as Pfeffer, that movements in constant light continue to proceed with equally large amplitude, provided that the nodes are surrounded by black cotton padding. When he took such plants to the mine shaft and placed them under entirely similar conditions, the daily movements became gradually smaller; finally they stopped entirely. However, the leaves remained able to react. /32

Cremer sees no chance to explain this remarkable phenomenon, and fortunately he does not venture one or another hypothesis, for he concludes:

"Electricity does not come in question for the development of sleep movements. We are dealing with a factor as yet unknown."

Cremer clearly considers nyctinasty not as an autonomous movement, but as an aitiogenous one, caused by an as yet unknown stimulus.

It is unfortunate that there are a few inaccuracies in Cremer's work. When we look at his curves which are reproduced, we see that the indicator made movements on the paper which are theoretically impossible; or the curves were later erroneously elaborated. For instance, whenever the indicator moves quickly up and down, an arc of a circle originates which is called time line. The curve described by the leaf intersects this time line at the upper side (lowest position of the leaf), and then withdraws more or less quickly from the time line (upward movement of the leaf). Now, according to Cremer, the leaf curve slopes from above obliquely downwards to the time line, which is an impossibility. This deviation is so strong, and it occurs so often that it can be no accidental mistake, or error in drawing (see for instance his curves 19, 20, 26 and 28).

233

I would also like to mention a remarkable number of books by J. C. Bose [7] from Calcutta. These are relatively little mentioned in the literature, which can undoubtedly be explained by the fact that Bose mentions only facts, and shows very few curves. Moreover, he is not always up to the level of newer publications which have appeared in Europe. But the instruments which he invented for recording, mostly with the use of electricity, all kinds of phenomena of plant physiology are very ingenious. Nyctinasty also attracted his attention, and through his tests he comes to the following conclusions.

The leaves of Mimosa do not fall asleep in the evening because it becomes dark, but rather as a sequela of the persisting light during the day. They recover during the night aided by a now oppositely directed aftereffect of the light, "consequent on the storage of internal energy by the day's illumination."

However, this is in addition to periodic impulses of the entire plant which have the same amplitude as light periods. The peripheral tissues of the plant are supposed to contract as a result of the day, and then press the water "into the central reservoir." At night then, a reverse movement takes place, so that "there is a periodic inflow and outflow, a diminution and increase in tension, and these variations of tension are indicated by the periodic depressions



and erections of all motile leaves synchronously."

As we look over the literature quoted, it seems that the opinions on the causes of nyctinasty are very much divided, so that Jost in 1923, in that well-known textbook of physiology by Benecke [3] and Jost, was able to remark that it is very difficult to decide whether sleep movements are caused by heredity, that is, they would be autonomous (Jost talks of endonomous movement), with the light perhaps acting in addition as a regulator; or that they should be entirely caused by external stimuli. Which of these hypotheses is true must still be further investigated.

## CHAPTER II

### Personal Investigations

#### A. Method of Procedure

In my first tests, I used the method described by Pfeffer in 1907 [29] /34  
for orientation. Briefly, it amounts to this (Figure 1). A wire is fastened to the blade; it is firmly affixed to a small straight glass rod at the upper end. This in turn is fastened to a small wire which is clamped between two corks. When the leaf sinks, this rod is supposed to hinge at the point of suspension so that one end moves upwards. When this rests on the treated paper of the kymograph drum, a line will be recorded. After a few days the paper is taken off the drum and fixed in shellac. When the indicator is moved rapidly to and fro, before the paper is removed, we obtain an arc of a circle, the radius of which can be calculated. The diameter is found by holding the indicator horizontal and rotating the drum rapidly. If the rotation time of the drum is known, we can indicate time lines, i.e., 12 o'clock noon and 12 o'clock midnight, with these data. By regulating the suspension point of the indicator, we can make the amplitudes as large as we want. The largest amplitude which Pfeffer obtained was 70 mm. He worked with sprouts of *Phaseolus*, while I found a particularly fine and very responsive subject in the primary leaves of a papilionaceous plant which is used in the tropics for green fertilization, i.e., *Canavalia ensiformis* D. C. In about four weeks, strong, large plants grew powerful sturdy primary leaves, averaging 15 cm long and 15 cm wide. The sleep movement is very intensive in these leaves. Even in the winter, when there was very little light, the sleep movements hardly decreased. Of course, young shoots which developed between the leaves and from the axils /35  
of the cotyledons were regularly cut away.

Since I wanted to have my curves larger, the indicator had to make considerable movements on the drum with the result that the curves became less clear. In the sleep position of the leaf, the indicator not only moved upwards, but the thread, AD, was also pulled backwards and upwards as a result of which the indicator often ran off the drum, and no line was recorded. In addition, because

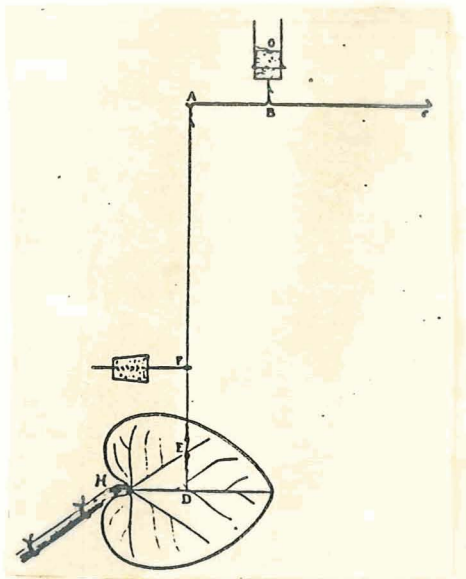


Figure 1: Improved method of Pfeffer. Explanation in the text.

the writing surface of the drum is not flat, but a bent surface, considerable deviations develop when the leaf is at its highest and lowest position. If the indicator continues to scribe still another line, then it happens that when the line is horizontal, it does not scribe with the point, but with a wider portion, which causes a very wide line.

Therefore, I started to make the following changes. In order that during great amplitudes the pull thread, AD (Figure 1) remains vertical, I attached a small glass eyelet, as low as possible, through which wire was threaded. When a strong bend took place, this wire bumped against the eyelet, and curved, as a result of which FA remained unchanged in position.

Pfeffer attached the pull and suspension threads firmly to the indicator. To get a more supple movement, I made a small bend in the indicator where the threads were hung with a small loop (Figure 1 at A and B).

To be able to quickly set up a plant or to change it, I fastened a small wire with a loop to the leaf, and connected this with the pull thread proper by means of a very thin S-shaped small glass hook (E).

Finally, I fused a small piece perpendicular to the point of the indicator.

/36

In this way, the indicator continues to write a proper line, even at the greatest divergence, which is the same everywhere. I used thin fine silk for the threads, sometimes also a very thin cotton thread, but only for the tests in the dark chamber, at constant temperature and humidity.

My kymographs, which are able to operate for a week, consist of a copper cylinder, 15 cm high and 10 cm in diameter, and are operated by the precise clockwork of a self-recording hygrometer. The correct level can be set with a small level on the drum and with adjusting screws. The smooth kymograph paper is placed wet around the drums, and then very evenly treated with the equipment of Zimmermann (Leipzig). Each day a specific hour is indicated by a small dash line on the curve (every day a different hour to prevent periodicity). I removed the paper after a week and fixed the obtained line in a clear shellac solution. With the help of carbon paper, I traced the curve on ordinary drawing paper, and worked it out in the already outlined manner.

Then I searched for a method which did not have the disadvantage of a cylindrical writing surface and curved time line. After long experiments and repeated improvements, I came to the following arrangement, which I used from then on. We see a schematic drawing of this arrangement in Figure 2. An instrument maker

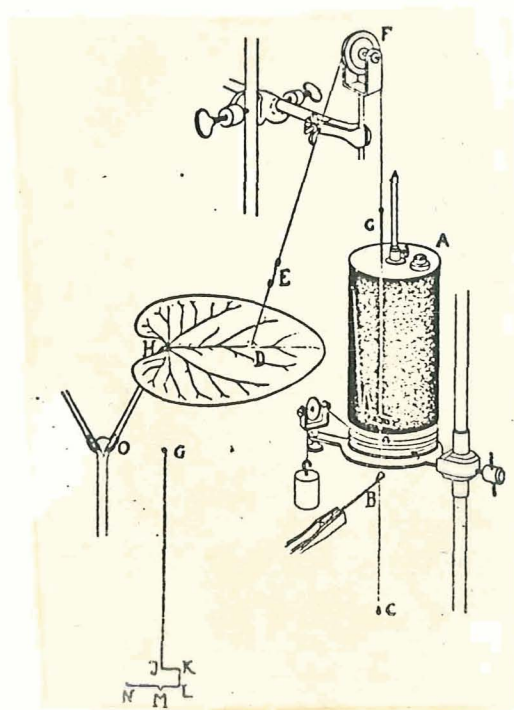


Figure 2: Schematic outline of the method I used. Leaf in diurnal position. Explanation in the text.

manufactured small, extremely easily running, stainless pulleys (F). A silken wire is run over the pulley, which is fixed in the clamp of a tripod stand, which is attached (E) with a small glass hook to the leaf in the manner already mentioned, while on the other side the glass indicator is hung in a loop.

This indicator is specially shown in the figure. I made it from a solid glass thread  $3/4$  mm thick. The GJ section is  $12-1/2$  cm, KL is 10 mm, and LMN is 20 mm. There is a bend at M on which the MC thread, which is  $\pm 24$  cm long, is suspended, and on whose lower end hangs a small 100 mg weight. This thread, MC, must fit exactly into the extension of GJ if the indicator is to hang precisely vertically. At N a very small part of the point is bent perpendicularly in order to obtain a quiet line, so that the upper part of the indicator will not touch the drum. Finally, for the reduction of possible swings of the weight, the MC thread passes from here through a glass eyelet (B) which is fastened between two half corks in the clamp of a tripod.

After the plant is placed in the correct position, the kymograph is pushed up until the point of the indicator touches the drum. In order to obtain a regular line, the indicator must rest against the paper with some pressure. This can be obtained by twisting the thread several times, by rotating the indicator a like number of times. The curves obtained in this manner are very regular, and clear, for regardless how large the leaf movement might be, the indicator always presses evenly against the drum. Since the indicator always moves in a plane on the drum, we obtain, by moving it rapidly to and fro, a line perpendicular to the line that is scribed when, at a standstill of the indicator, the drum is rapidly rotated. The horizontal line must be determined before the lines are removed from the clockwork, for the fixation of the obtained curve. With the help of time marks which we have made every day, the time ordinates can then be vertically plotted.

The most important factor for the successful use of this method is an extremely precise construction of the indicator, so that the vertical and horizontal parts of it (except the outermost point) will lie exactly in one plane, and all bends are well made vertical. After a little practice, this is achieved very soon.

Initially, in imitating Pfeffer, the stem and the stalk of both leaves are fixed, but later tests showed that the stalk movement is extremely small, and I stopped a further fixation of it. I will return to this later on. I placed plants and pots in a glazed tray with water, taking care that this never dried up entirely. The trays were refilled at very irregular times. The first tests were made with seeds which originated from different botanical gardens. Except for the difference in size, sprouts which developed from it were alike in appearance, and gave similar curves at recording, of course leaving out of consideration any individual differences. In the autumn of 1924, Prof. Dr. Stahel from Paramaribo was kind enough to send me a large consignment of Canavalia beans, for which I again express my sincere thanks. Initially, it was impossible to bring these beans to germination. They swelled up, but before the thick episperm was entirely off, they started to rot because of an attack by bacteria. One of the gardeners, however, who always nursed my plants, found a way: he painted the beans with a small artist's brush plunged in motor oil before he planted them. As long as the young germ was not yet above the ground, he did not water them. And indeed, from then on, all seeds came up nicely without a single exception.

The West Indian plants were much more robust and more vigorous than the European plants, which had to be ascribed to the more favorable circumstances under which they were growing, in comparison with the European plants, which had to be nursed in boxes. But regardless of how different the origin might have been, the daily movements of the leaves were for the most part similar to each other, as can be seen, for instance, by comparing Figure 4 with Figure 5. I used the leaves only after they did not grow any more, which is about after 4-5 weeks, and I never continued a test until the leaves started to look poor.

It is astonishing how much these sprouts could tolerate. After fourteen /39 days in constant darkness the leaves were still as fresh as at the start. I often kept plants which became too old for me to experiment with in a dark corner of the chamber, or of the greenhouse, until the leaves started to become yellow. Yet, their movement still continued rather well.

The experimental greenhouse in which I worked was very large, and it had much light on all sides. It could be kept fairly warm in the winter, even though the temperature was naturally not constant.

With regard to humidity, the greenhouse in which I worked was thoroughly sprayed twice a day.

The times at which this happened were always precisely noted by me or by a gardener. But I could never find any sign that after, and because of, the spraying the position of leaves changed.

My tests with artificial light were made in a dark chamber. Illumination was obtained by means of two Philips 500 watt sunlight lamps. These lamps hung in a large glass bell. Each bell rested on a cork ring in a somewhat wider bell. Cold water continuously flowed through the space between these two bells, so that the plants which were kept between the two lamps were always exposed to a constant temperature. I threw a small grain of mercury bichloride into this water from time to time, so that no algae could develop. Each of the bells stood in a sink through which the water drained off. The lamps were placed so high that the light fell upon the leaves right from above. The distance between the two lamps was 85 cm, between which the plants were set up with the tripods and the kymograph, which was often very difficult. Most of the time I recorded two plants at a time on one drum, so that four plants were recorded at the same time, which greatly increased the reliability of the results. The following arrangement was made in order to keep humidity constant. A copper T-pipe was mounted just above the bottom of a very large water bath. The horizontal portion of the pipe was 30 cm, while on the lower side a very large number of small holes were bored. The water bath stood on a small electric stove, and this again on a tall, small table. A piece of jute rag was placed under the T-pipe and fastened on a wood stand which sloped at a 45° angle. With the aid of a tap which was placed on the T-piece, the speed with which the water streamed from the holes could be regulated in such a way that the water in the water bath reached a high temperature before it dropped on the jute rag. As a result, the rag was constantly moist, and, because of the warm water, steam ascended from it incessantly. The water then ran slowly down the rod on a freestone slab (which was placed in the room for tests which had to be carried out free of vibration), and from there through a groove between this slab and the boards into the sand cellar, located under the building. The water around the lamps increased the humidity so very little, that it was not noticable whether the lamps were burning. For that matter, it was maintained

/40



at constant flow. With this arrangement, the self-recording hygrometer showed a humidity of 65%.

To keep the temperature constant, the Kipp firm of Delft installed a thermoregulator, which kept the temperature constant within  $1/2^{\circ}\text{C}$ . A second, small electric stove was switched on at the smallest drop of the temperature, but as soon as the required  $25^{\circ}\text{C}$  was reached, the stove switched off again. The temperature remained constant in this way even on the coldest days in the winter; only on a couple of very hot days in July, 1925, did it rise to  $29^{\circ}\text{C}$ .

In this dark chamber, there was no gas pipe.

### Description and Discussion of the Tests

During the discussion of the various curves, many terms are repeatedly mentioned which I will discuss here in more detail.

In Figure 2, we distinguish on the leaf:

The stalk node (O), which is the node at the transition of the stalk into the stem. As a result of its movement, the stalk droops in the evening, and moves up again in the morning. The amplitude of this movement however is very small.

The blade node (H) connects the stalk with the blade; this makes the blade droop in the evening and move upwards the next morning. In the first case we speak of the nocturnal position (Figure 3), in the second case we speak of the diurnal position (Figure 2). Instead of nocturnal position, I also use the word sleep position, without trying to hint about a connection with animal sleep.

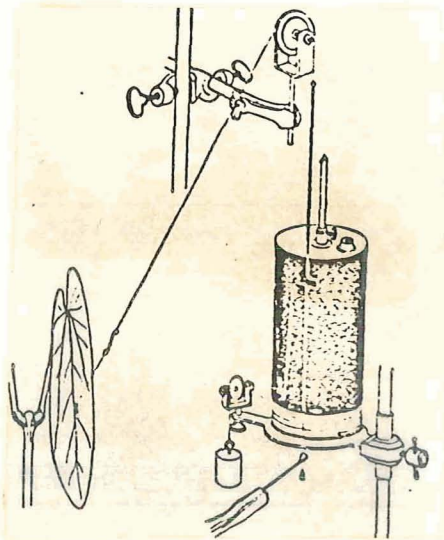


Figure 3: Schematic outline of the method which I used. Leaf in night position. Explanation in the text.



In studying the reproduced curves, we kept the following in mind:

a) when the leaf droops, the indicator moves upwards, so that the upper side of the curve is in agreement with the sleep position of the leaf.

Thus, the lowest portion of a curve is the movement of the leaf into the diurnal position;

b) midnight is always indicated by a thick black line.

Twelve o'clock noon is indicated by a thin line with a date;

c) for tests in the dark chamber, a dark period is always indicated by a gray tint. The "natural night" in the chamber can be found always by the thick line.

It happened repeatedly during a forceful movement of the large *Canavalia* leaves, that in the sleep position the leaf passes through the vertical, until the tip is finally pressed against the stem, sometimes with great force.

During the day, on the other hand, the leaves frequently move upward with such force that they point obliquely upward. The amplitude of these movements is frequently more than 90°. As a result the indicator several times ran off the drum from above or from below, which shows in my curves as a dotted line.

Five strong ribs run through the blade. Since I shall use these as one, /42  
I shall call the middle one the midrib (HD) (Figure 2).

I fastened the pull thread to this midrib. Initially, I used pure gum arabic, but when it seemed that this gets loose in the great humidity of the dark chamber, I made a small hole on both sides of the midrib, and tied the pull thread around the rib, protecting it with a small piece of cotton. It was safe to do so, since it was shown in a test that such a wound stimulus has not the slightest influence on movements. For that matter, Pfeffer also used this method with the much more delicate *Phaseolus* leaves.

The distance from the blade node to the pull thread is designated HD. In order to be able to compare the curves obtained as much as possible, I tried to keep this distance always the same. HD=7.5 cm in the European plants with their rather small leaves, while in the powerful and large Surinam plants, HD=8 cm.

To obtain the best possible graph of the leaf movement, I took care at the start to ensure that when the leaf was in the horizontal position, the pull thread was at a  $60^\circ$  angle to the blade (to save space, in Figure 2 the blade is drawn too close to the drum, with the result that the intended angle is too large there). Of course, for all that, a leaf movement in the highest position continues to give a somewhat larger swing than when it is in the lowest position.

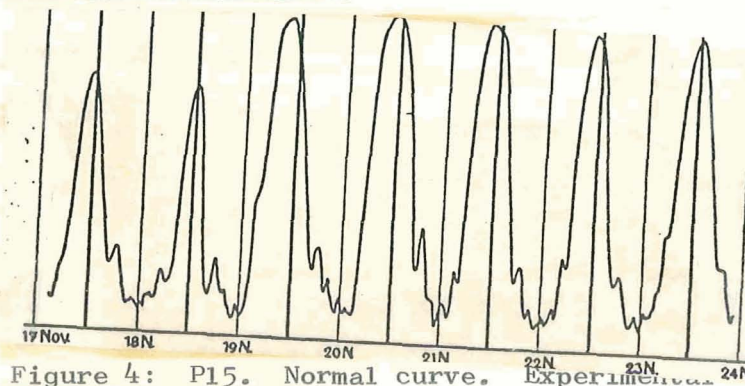
I converted all times to standard astronomical time during the months when the daylight saving time was in effect.

I had a large number of sprouts always ready for use, from which I selected the finest and healthiest specimens at the start of a test. The one leaf of such a plant I designated P with serial number, the opposite leaf was designated P' with the same serial number. Almost all tests were made with two, often even with four plants at the same time, in order to make the result as reliable as possible, and to eliminate possible individual deviations. I often made the same test several times to obtain absolute reliability. Here, I will always reproduce only one curve, selecting the one which shows the results obtained in the finest way. But I would like to repeat once more that from a number of curves with more or less opposite findings, I did not select the ones which agree best with a theory, but that different plants showed the same phenomena.

All figures are reproduced at one third their actual size.

## B. Tests in the Greenhouse

### 1. Discussion of Normal Curves



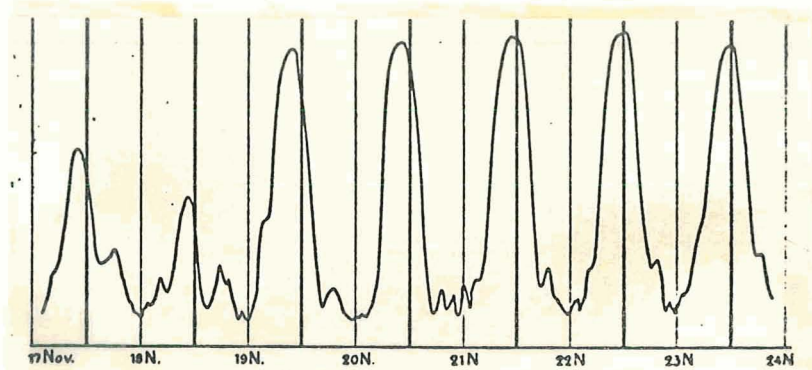


Figure 5: P16. Normal curve. Experimental greenhouse.

We have seen through the literature review that the great question existing with respect to nyctinasty is whether the movements are autonomous or aitiogeneus. I would now like to collect some new data, whereby a conclusion could be made /44 in favor of one or the other concept. Furthermore, I would like to pursue whether they carry out their movements independently.

For this we must first look at the curves, which two different leaves of plants from seed coming from Innsbruck, described during a week in the experimental greenhouse. We can see these "normal curves" of P15 and P16 in Figures 4 and 5. It seems that the lowest position is always reached about 12 o'clock at night; thereafter the leaf quickly moves upward and about 4 or 5 o'clock in the morning has already reached a very high position. This is followed by a more or less small return, and at approximately 12 o'clock noon the leaf has reached a peak position. It often passes through the horizontal at this time, and stands obliquely upwards. In the diurnal position then, a number of swings are carried out which Pfeffer considered as "autonomous" movements. Yet, I could often observe that when I inspected the plants for one reason or another, especially when they stood in the dark chamber, I was able to observe that more small swingings developed. Stoppel [48] also mentioned that her curves were less even when she touched the plants a few times. As we shall see later, it is easy to revert the movement of leaves by creating darkness during the day, and by providing illumination at night. Such plants are in the highest position when the plants in the greenhouse are in their sleep position. But then also, small swingings are carried out by the first

mentioned leaves. These swings are apparently not a sequela of certain external conditions, but are caused by the diurnal position itself. This is apparent from the fact that in constant light and darkness, when sleep movements continue normally, the small swingings recur when the leaf is in its highest position. It is not improbable that the weight of the blade is the main cause; for its static moment, i.e., the node is the largest when the blade is in a horizontal position, then in the node the greatest force must be applied. Whenever the turgor slightly decreases in the node because of slight disturbances, or by respiration, evaporation, or other metabolic functions, it must be felt mostly in the horizontal position of the leaf. What is more, both Pfeffer's and my methods show that in this position the recording is the most sensitive. In addition, one plant is supposed to be more sensitive than the other; therefore, the small swingings often show very great individual differences. /45

The smaller night peaks on 17 and 18 November are peculiar for both plants. The cause of this is difficult to establish. I suspect that temperature played a considerable role here. The days preceding the one on which the test started were very cold, which should have had some influence, because the plants were still in the nursery greenhouse, which had little heat, while the greenhouse where the tests were made was much warmer (during the day up to 25° to 30°C), so that the amplitudes soon become much greater. It is worthy of note that during the entire week of 17 to 24 November the days were dark and the weather very fall-like, with a moment of sunshine now and then. The decreased illumination and unfavorable time of the year appear to have very little influence upon nyctinasty. I assume that all this time assimilation must have been extremely small. In contrast to the statements of Cremer [11], I could not find the slightest difference between winter and summer curves. Even the plants which stood in the dark chamber had almost similar curves to the ones reproduced here, no matter what season of the year it was, and, oddly enough, even for the reversal or prolongations of the period. Compare Figures 19, 29, 30 and 36.

The drop and rise of leaves occurs rather rapidly and very regularly. In the majority of cases the lowest position is reached between 10 and 2 o'clock at night. Deviations from this occur now and then, but then also with most plants at the same time, so that we must take as its cause one or another deviation in the external environment.

If we compare these times with what other authors gave for *Phaseolus*, we find quite a few differences. In the case of Pfeffer's [29, 31, 23] curves, the lowest position falls always before 12 o'clock at night. On /46 the other hand, Stoppel found it to be between 3 and 5 o'clock in the morning. With Cremer [11], it is between 3 and 6 o'clock in the morning, while Schweidler [37] and Sperlich could find no definite time, but they indicate it between midnight and 11 o'clock in the morning. Bose [7] finds the lowest position for *Mimosa* around 6 o'clock in the afternoon, and the highest around 7 o'clock in the morning.

Pfeffer's statement comes in closest agreement with my findings for *Canavalia*.

## 2. Tests with an Artificial Leaf and with a Covered Blade

The question arose whether in the case of nyctinastic movements, we could also speak of a perceptive blade and a responding node.

For this I removed the blade from a fine, straight leaf, with the exception of a very small part, at the blade node. To this I fastened an artificial aluminum blade which was exactly as heavy as the removed portion, while the pull thread was fastened at 7.5 cm distance from the node. Although on previous days this leaf described a normal curve, the next day the movement had already stopped; the indicator scribed a horizontal line. The control plant reacted normally. The node on which the artificial leaf was fastened looked perfectly healthy and turgescient, while after a few days it seemed that its stalk made entirely normal daily movements, which were exactly as large as in the control plant. However, when I repeated this test once more later, the artificial leaf did not move as quickly, but on the first few days the movements were still small, in the usual daily rhythm. For reasons to be mentioned later, it occurs to me that this is the result of the strong wound stimulus, since when the entire ribs are removed, so much water is supposed to accumulate in the node that movement is made impossible.

In order to avoid the objection about wound stimulus, I changed to wrapping the blade with paper. I let all four leaves, P13, P13', P14 and P14', from two strong plants, write normal curves for a few days. Then, on November 3, /47 the whole blade of P13' was wrapped in a shining white kymograph paper, and



that of P14' in dull, dark paper such as is used in photography; the papers were of exactly the same weight. The blade nodes remained uncovered. We see the results in Figures 6 and 7. The leaves dropped equally deeply at night, but during the day they did not rise as high as usual.

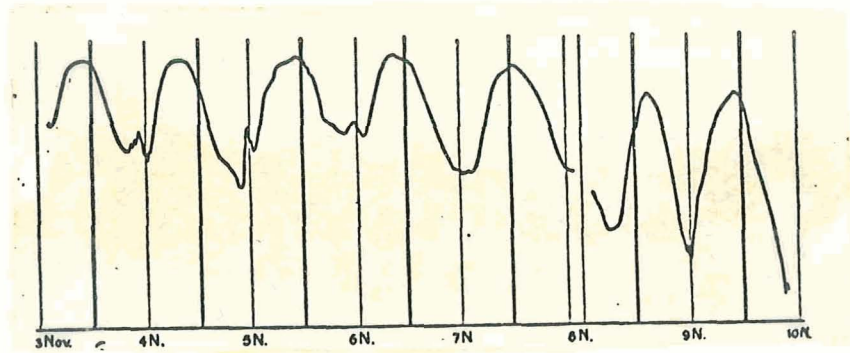


Figure 6: P13'. Blade in white paper. Experimental greenhouse.

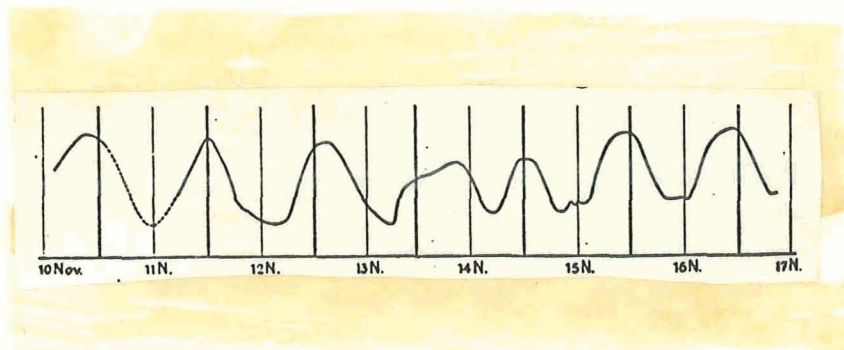


Figure 7: P14'. Blade in black paper. Experimental greenhouse.

The small swingings in the diurnal position almost disappeared, since the leaves were only at an upward angle because of the weight of the paper. It seems that one cannot speak of a different influence of white and black paper. The opposing leaves P13 and P14 served as control, and showed no deviation. I removed the paper on November 8, and very soon the movement was normal again.

Two days later I reversed the test. Now, the black paper was wrapped around P13' and the white around P14', again with the same result. The movement was /48

certainly somewhat smaller than normal, but it was not deviating from it. Figures 8 and 9 show that there was no different effect from white and black paper.

The following is peculiar. Figures 8 and 9 show less low a peak during the night from 13 to 14 November in both plants, but the two control plants also drooped less low as on other nights. Thus, the same factor which hindered the normal leaves in reaching their deepest position made itself felt on the leaves wrapped in paper. All these days were very cold and dark, so this must account for this phenomenon. We can see again, even with these leaves wrapped in paper, that the lowest position is reached almost always around 12 o'clock at night, the highest around 12 o'clock noon.

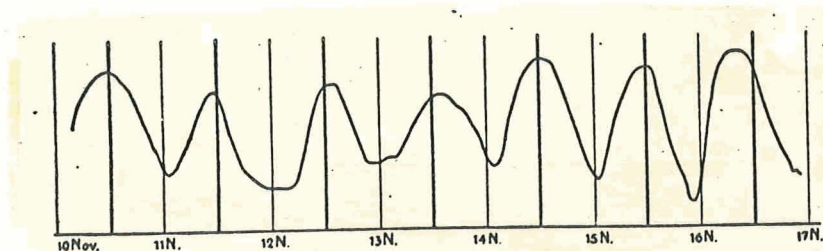


Figure 8: P14'. Blade in white paper. Experimental greenhouse.

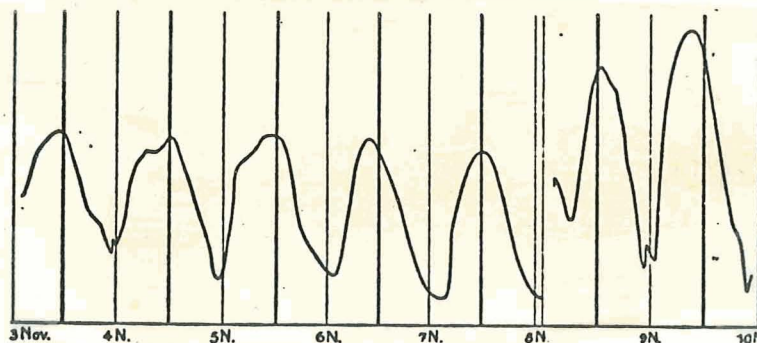


Figure 9: P13'. Blade in black paper. Experimental greenhouse.

There are now three possibilities. The unwrapped nodes have perceived the alternation of day and night; thus they are simultaneously reacting and perceiving, the opposite normal leaf had induced the movement in the covered leaf, or, there is a still unknown factor at work which easily passes through the white or black paper. That it should be aftereffects seems to me out of the question, since the movements lasted a whole week and they certainly did not decrease in intensity. For that matter, the already mentioned lower peak in all four leaves argues against this possibility also.

/49

Pfeffer [32] found something similar. As early as 1915, he reports: "Nevertheless, the plant reacts in such a way to changes in illumination even when only the node is exposed to light, but the blade and all other parts are kept in constant darkness." It is unfortunate that he does not give anything further on the course of the curves, and that none of them are reproduced.

### 3. Tests with Cut Leaves

A publication of L. G. Rommell [34] in 1918 mentions that root pressure has a clearly expressed diurnal period which also seems to be present in plants which are raised under completely constant conditions in constant light. It is not unthinkable that this periodicity should have a more or less great influence on the sleep movements. Generally, it seemed to be desirable to investigate how nyctinasty is related to the entire metabolism of the plant.

By working with cut leaves, I could eliminate not only the influence of the entire root system, but also an induction possible from another leaf. My first method was to cut off the leaves under water, placing wet cotton pads on the wet surface, and then fasten the stalk on a glass rod. This rod with the leaf on it was then placed in a test tube with water; the tube was fastened to the clamp of a tripod stand. This tube was at such an angle that the stalk made the same angle with the vertical as when it was still on the plant. Thus the position of the leaf did not actually change. By this fastening on the rod, it was possible to take the leaf any time from the tube for cutting off the wound surface. When the leaf was replaced in the test tube, I was assured of the leaf having the same position as before its removal. Later, I used a method which led to quicker results. I took a few small, 250 ml bottles which also had an opening at the bottom. A rubber plug was placed in this bottom opening through which a glass tube passed, somewhat thicker than the stalk which was bent just so far that the stalk therein had the same position as on the plant. This tube reached almost to the neck of the small bottle, so that, when it was full of water, the stalk had sufficient moisture. This made daily refilling unnecessary, and moreover, took up much less space on the table than a test tube in the tripod.

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In order to prevent, to the extent possible, any metabolic disturbances, I placed the leaves in Knop nutrient solution.



For all tests I made there was one (sometimes two) control leaf present which scribed the normal curve, while each leaf used for one test or another always recorded the normal curve for at least four days. This eliminated accidental deviations, and I was sure that no leaf, which showed abnormal movements in any way, was used in the tests. Of course, I did not reproduce the normal curve every time. For orientation, I cut off a good leaf from a plant, and recorded its movements. To my disappointment, the movements were already considerably less after 24 hours, and the leaf started to become limp. When the movements practically stopped after two days, I assumed that wound tissue had formed which obstructed the water access, and, as a result, the sleep movements. I had left a large portion of the stalk node on the stalk when cutting the leaf of the plant, since I still did not suspect what a great role this would play. I cut off a considerable piece of the stalk, to remove the supposed wound tissue, whereby this piece of the stalk node disappeared. The next morning I was surprised by the change which had occurred in the leaf. It was almost normally turgescient again, the yellow spots had almost disappeared, and there was considerably increased movement. I noticed on the previous day that there was no wound tissue to be found at the cut piece of stem; I could only find a few black dots on the cut surface. Therefore, it seemed improbable to me that its removal should have had such a strong influence on the leaf. /51

Therefore, I repeated the test once more with increased care. Plants P16 and P17 had recorded very nice, normal curves, despite the dark, cold weather. On December 8, I cut off the two leaves from P17, in such a manner that P17' had no stalk node, and P17 had still one stalk node. I took the two leaves from a single plant on purpose, in order to avoid individual differences as much as possible; in addition, I took the most powerful leaf with node of these two, and somewhat less vigorous one without node. P16 was the control plant during this test. In Figures 10 and 11 we see the great difference. P17', without the stalk node, continues to move practically unchanged. We could not see from this curve that it was not a whole plant, but a cut leaf. P17, on the other hand, with the node, immediately became considerably less, which shows up well from the part of the normal curve which was reproduced. The out-

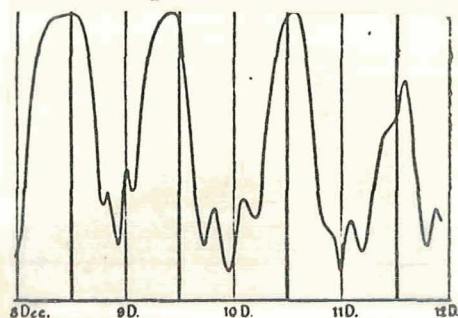


Figure 10: P17'. Cut leaf, without stalk node. Experimental greenhouse.

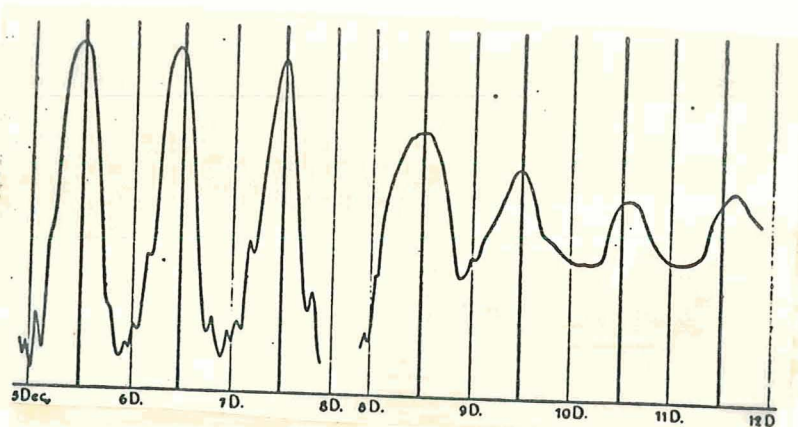


Figure 11: P17. From 5 to 8 December: normal curve. From 8 to 12 December: cut leaf with stalk node. Experimental greenhouse.

side of this leaf has also markedly changed. P17' is green and turgescient. /52  
P17, however, is limp and has yellow spots, which have expanded considerably along the midrib after four days. On Plate 1, we can clearly see the difference between the two leaves (taken 12 December, in the afternoon around 2:30).

On 12 December, I cut away the stalk node in P17, and although it was a very dark day and the weather was cold, the leaf immediately recovered; on the same night it drooped much lower than was the case until now. In the following day the movements returned to a considerable extent, the leaf again became turgescient and the yellow spots were smaller on the next day; finally they disappeared. P17' continued to move with vigorous amplitude during this time. Plate 2, taken on the same time of the day as Plate 1, shows P17 after its recovery. The change which occurred in the leaf comes out well on both plates.

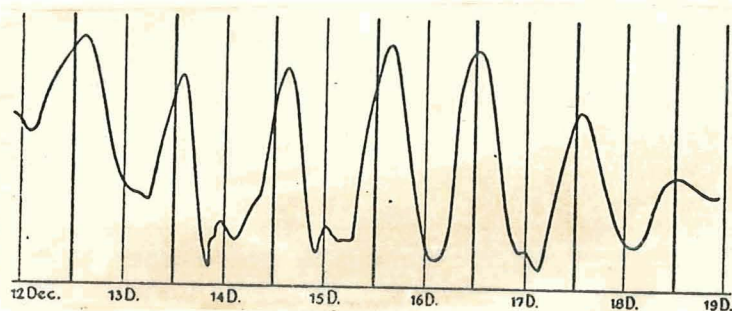


Figure 12: P17. After removal of the stalk node. Experimental greenhouse.



Figure 12 shows the curve which the leaf had described during the week. Both P17 and P17' started to show signs of deterioration on 18 December. Thus, the leaves continued to move normally well over 10 days. I made a similar test in the dark chamber also. Although this was not possible until February of the next year because of certain circumstances, I will report the results here. I took four leaves of two plants which were raised in the greenhouse. One leaf with stalk node and one leaf without stalk node were used from each plant. The results were similar to those obtained in the greenhouse. In Figure 13, we see the curve of P28 without, and of P28' with, the node. During this test, temperature, humidity, and illumination (2 lamps of 1000 candles each) were constant. In P28' the movement, except for the smallest undulations, ceased by 12 o'clock, as opposed to the other leaf which shows, it is true, not so vigorous but still distinct periodic movement, although it was in constant light. The leaf with node in the greenhouse at least still showed a small movement, which was missed here entirely. Another difference from the greenhouse experiment is that in the dark chamber the leaf with the stalk node did not get any yellow spots, but dried up entirely. First it became limp, then the top started to become dry until finally the whole blade became dry and started to curl along the edges. The leaf pulp along the midrib remained intact the longest. The color of such a leaf is dark green, and, I repeat, without a

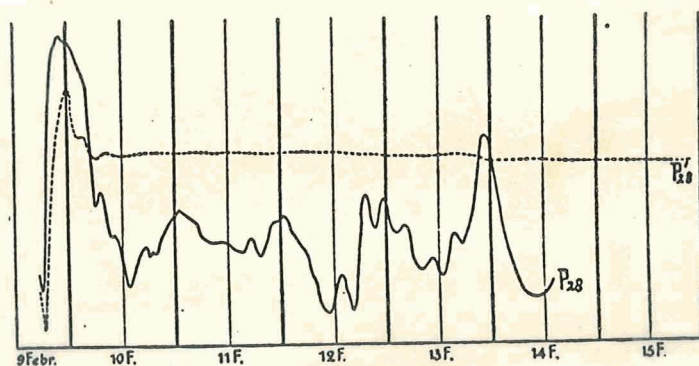


Figure 13: P28 (solid line) without stalk node. P28' (dotted line) with stalk node. Dark chamber.

trace of yellow spots. To be certain, I repeated the process with two more leaves, without recording the movements. The process always had the same course: in the greenhouse yellow spots, in the dark chamber rapid drying and dark green color. Undoubtedly we are dealing here with an arrest of water, due to the

presence of the stalk node. As soon as this is not cut away, the leaf recovers, both in the greenhouse and in the dark chamber, only here not as completely. <sup>54</sup> They always remain somewhat limp, especially when the process was permitted to go on for a long period. Finally, I took two leaves with stalk nodes, and two leaves without, P18-P22, and P18'-P22' respectively, and every day I weighed these four leaves to track down the speed of exsiccation; the leaves were kept in the dark chamber under constant conditions, after the stalks were carefully dried up. Figure 14 shows once more the difference in the appearance between two such leaves, i.e., P18 and P22'.

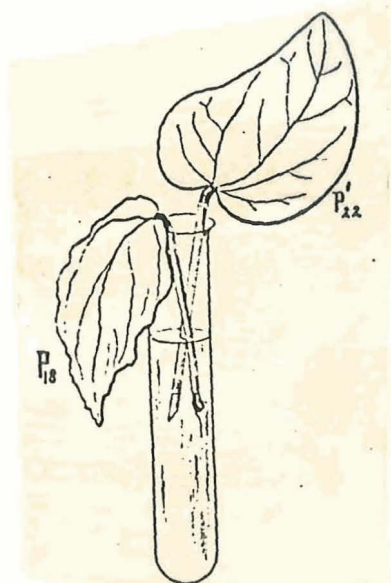


Figure 14: P18 with stalk node.  
P22' without stalk node.

Here is the obtained table:

	P18	P18'	P22'	P22
2 Feb	1,705 g	1,420 g	1,070 g	1,140 g
3 Feb	1,189 "	1,390 "	1,104 "	0,995 "
4 Feb	0,894 "	1,445 "	1,116 "	0,994 "
5 Feb	0,865 "	1,405 "	1,114 "	0,831 "
7 Feb	0,798 "	1,494 "	1,130 "	0,699 "
9 Feb	0,796 "	1,508 "	1,150 "	0,770 "

Commas represent decimal points.

We notice that the normal leaves continue to gain in weight, while the others deteriorate rather quickly.

In order to find the cause of this peculiar behavior of the stalk node, I then investigated whether I could observe some anatomical difference on microscopic sections. I did not succeed in this as yet; I did not have enough time to take this up in more detail. Yet I am certain that something will come to light, especially in connection with a peculiar fact which I observed accidentally. I set a number of plants to be used for tests in a corner of the dark chamber, /55 and no further attention was paid to them. After a few days, I found one morning that the two leaves had fallen off and were lying next to the stem. At closer inspection, it seemed to me that the larger part of the node was still firmly attached to the stalk. A layer of cork had formed on both fractured surfaces on the place where the node of the stem was detached. This leads me to assume that when a leaf with stalk node is cut and put in water, the cork layer begins to form very soon; thereby, water access to the leaf is blocked. Closer investigations may determine whether this assumption is correct. Meanwhile, this question has little to do with the matter of nyctinasty, and I shall not pursue it any further.

The tests with cut leaves certainly show that the sleep movements are entirely independently localized in the leaf. Other processes which occur in the roots and in the stem therefore have little or no influence on nyctinasty.

While I made these tests, I was also carrying out other investigations in the dark chamber, which were especially concerned with the influence of illumination, and by its very nature form a united whole in some respects. Therefore, I shall discuss them after the greenhouse tests.

The investigation in the greenhouse was a search in various directions in the hope that a phenomenon would present itself, which would throw some light on the still so confused and complicated picture of these sleep movements. Although many of these experiments did not produce any results, there were a number which I find worthwhile to mention here.

I wanted to investigate to what extent the movements of the nodes were influenced by humidity. For this purpose, I placed two leaves upside down into a small dish with water so that the blades were partly under, partly above, the water. The part which protruded from the water was greater than the immersed



part, so that assimilation was disturbed as little as possible. The stalks stood out above the water. The pull threads were fastened on the stalks, at a distance of 7.5 cm from the blade node. One leaf (P47') was put in the glass /56 dish so that the blade node was under water; the other leaf (P47) was placed such that the node was kept above the water with the aid of a small piece of stone placed under it. This small piece of stone secures the blade so that it could not shift during the test. Figure 15 shows the arrangement of P47' with submerged node. Figure 16 represents the curves which the two stalks P47 and P47' recorded from 12 to 19 March. The curves show that during the first few days the movement is indistinct, but thereafter it again shows the usual daily period, although with a smaller amplitude. The peculiarity is that

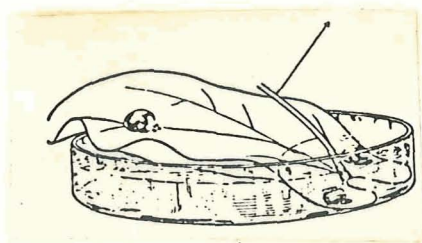


Figure 15: P47'. Explanation in the text.

only in P47', where the node is under water, the movement returns two days earlier. The assumption had been made that this node absorbed so much water that the movements would cease. As is shown in the figure, from March 16 on there is generally much agreement between the movements of the two stalks.

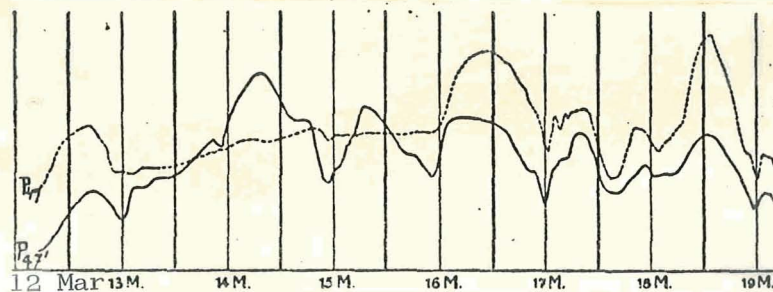


Figure 16: P47' (solid line). Blade half under water. Blade node under water. P47 (dotted line). Blade half under water. Blade node above water. Greenhouse experiment.

In a repetition of this test, I obtained the same result. The explanation of this is difficult to give: either the external stimulus departs by the water, /57 or it is perceived by the part of the blade standing out above the water, and from there is conducted to the node. Meanwhile it became desirable to investigate whether perhaps assimilation could have some influence on nyctinasty. For this

purpose two cut leaves, each on a kymograph, were set under two large glass bells. Each of these bells stood in a copper tank with two holes in the bottom, wherein rubber plugs were fastened with glass tubes. The bell is fastened airtight in the tank with a broad rubber band. With the aid of an aquarium pump, air, which was first separated from carbonic acid, was conducted through the glass bells, so that there was always a small overpressure. But neither in the greenhouse nor in the dark chamber under completely constant conditions could I observe a definite influence. The movement of the leaves is less, but that also happened when normal air was passed through. I had no opportunity to continue these tests in greater detail; in addition, I became convinced from other indications that the influence of assimilation on the sleep movements is not very large.

#### 4. Tests with Leaves under Water

Suessenguth [51], in his "Investigations of the variation movements of leaves," reports, among other things, that he kept leaves in salt solution to study its effect. (The leaves were kept in it upside down to prevent that liquid from getting on the ground.) Some leaves were kept upturned in usual water for control; their movements continued normally. I decided to make this test also. Cut leaves were fastened in the described manner in bottles, and then, with this bottle under water, placed in a large glass tank. The leaves were entirely immersed. A small section of the pull thread to the indicator ran through the water. The leaves quickly assumed their equilibrium position. As a result of the upward pressure of water they slanted upwards, and then did not make any more movement. This result was somewhat unexpected, in comparison /58 with what we found when we, as in the previous test, kept the node with a portion of the blade under water. I assume that the node was not able to move the large blade against the upward pressure of water.

One drawback was that bacteria developed very quickly, no matter how carefully I rinsed off the leaves. When I touched the leaves after six days, during which I renewed the water twice, I saw to my astonishment that the blades were sitting loose on the stalks; the nodes were completely full of slime by bacteria. I repeated this test in the dark chamber at constant temperature, humidity and also constant light, after I had made the bottoms and the tanks bacterium-free as much as possible under streaming hot water. The two leaves, P56 and P57,

were motionless in 24 hours, after they again first assumed the equilibrium position. Two days later, on the 8<sup>th</sup> of April, the temperature was 24°C in each aquarium at about 4 o'clock in the afternoon. In case of P56, without bumping or bringing the water into undulation, I put a large piece of ice in the tank with the result that the temperature gradually dropped, so that it was 16° around 4:34, thus 8° less. The ice was then pulled out, and the temperature started very slowly to climb to room temperature of +24°C (after 20 minutes, it rose only 1°). Both the rapid cooling and the increase in temperature were without the least effect on the leaf; the curve continued as a horizontal line. A few days later, I removed the lamps, and kept the leaves in constant darkness, but again without a trace of response. I do not need to reproduce the obtained curves, for they are pure horizontal lines, so that everybody can imagine their course very easily.

#### 5. Removal of the Upper or Lower Half of the Node

We have already pointed out in the literature review that there is more than one concept as to the manner in which the leaves react, when the upper or the lower side is removed from the node. Pfeffer [28, 29] assumes that the two nodal halves react on a stimulus in the same way, but with different speed; for, when he removes the upper half from the node, the leaves go upwards in the evenings. If the lower half is off, then they will droop. It follows from this that the tension increases in the evening in both halves.

Jost [21] and Schwendener [38], however, find that in all cases the leaves maintain their normal movement, which is only possible when the tension in the lower half of the node decreases in the evening; then, when this half alone is present, the leaf will still droop towards the evening. If, on the other hand, the upper half alone is present, and the leaf droops in the evening, then the tension in the node half must have increased. They conclude that there is an uneven response in the two sides of the node. Then comes Wiedersheim [55] with the solution: when half of the node is cut away up to the vascular bundles, a reaction as indicated by Pfeffer follows. When, however, the half is not cut away as deep, he finds the same results as Jost and Schwendener. Lepeschkin [23], in 1909, corroborated the conclusion of Wiedersheim. Canavalia behaves in this matter still in another way. The two leaves from a big, fresh plant

/59



were used. In P50 the lower half, and in P50' the upper half, of the blade node was removed in such a way that the white vascular bundle was just revealed. The wound surfaces were smeared with pure vaseline to prevent exsiccation. This cutting repeatedly showed that the lower side had much parenchymal tissue, which immediately showed water. The upper side is much tougher, and appears to have less water. Otherwise, the nodes are exactly constructed as *Phaseolus*, of which Pfeffer [28] gives a drawing in 1875. We also find a longitudinal and transverse cross section through such a node in his 1915 publication [32] on page 6.

As soon as the lower side in P50 was cut away, the upper half curved to such an extent that the blade went through the vertical and was pressed against the stem of the plant. As can be seen from Figure 17, it remained motionless in this position for a week; all reaction capacity had disappeared. P50', however, with only the under half of the node, continues to stand completely horizontal, with the only difference being that it droops a little more at night; during the day, however, it stands more or less obliquely upwards.

/60

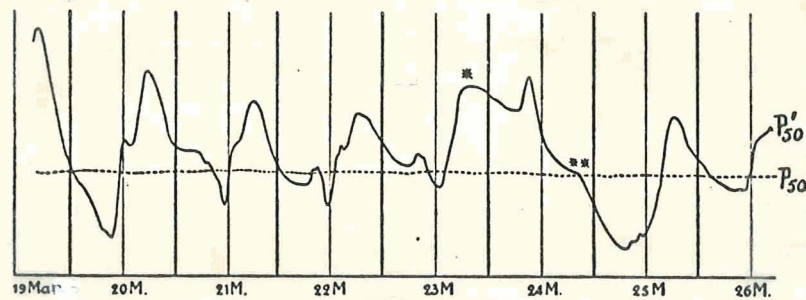


Figure 17: P50 (Solid line). Lower half of the blade node is removed. P50' (dotted line). Upper half of the blade node removed. Greenhouse experiment.

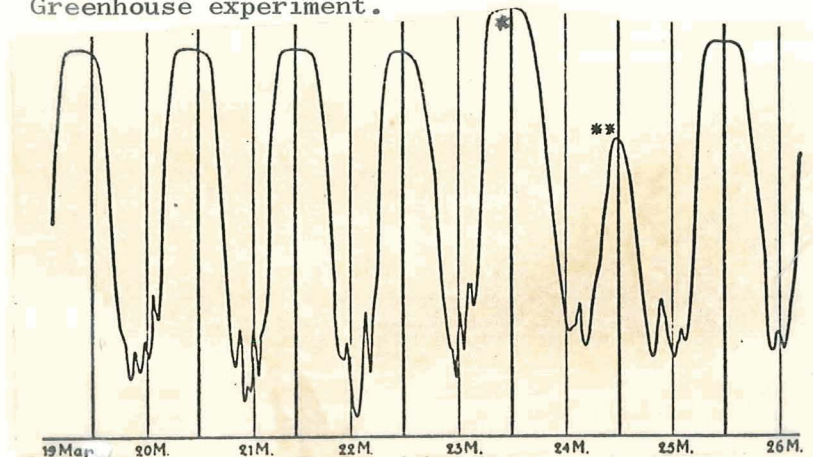


Figure 18: P51. Control plant. Greenhouse experiment.

In the same figure (17), we see the behavior of this leaf. The difference from the control plant P51 is, as it appears by comparing Figures 17 and 18, that the lowest position of P50', however small it is, now appears at 6 o'clock in the morning, while P51 is at the turning point at exactly 12 o'clock. I repeat, however, that the movement of P50' was also barely perceptible, so that, whenever we came into the greenhouse late in the evening, and all Canavalia plants were in deep sleep positions, those with only the nodal lower side appeared to be practically in a horizontal position. It is again peculiar that we see in the mentioned figures that P51 shows extremely good movement despite the very dark weather. During the night of 23-24 March, it dropped so deep that the indicator ran off the drum, but even P51' dropped somewhat deeper than usual in the same night (in the figures indicated by an \*). The following night P51 dropped very little, while P50' only shows a feeble indication, but nothing further of a night climax (indicated in the figures by \*\*). I repeated this test without recording, always with the same result. It can be concluded from this that the under half of the node shows much greater activity than the upper half. My results in this respect do not agree with either those of Pfeffer or those of Jost and Schwendener. When we assume that the upper half has rather great, but constant turgor, and the under half a periodically alternating tension, we can explain the curves of Figures 17 and 18. The turgor should decrease on the lower side of the node in the evening, as a result of an external cause, let us say, the light, to such an extent that the pressure of the upper half increases and it therefore presses the leaf down. In the morning, then, in such a normal leaf, the turgor will increase so much in the lower side that it becomes stronger than the tension of the upper side. If the upper half of the node is not present, then the leaf will certainly droop to some degree toward the evening through the decrease in tension in the under half of the node; but much less so, because now the pressure from above is missing. If there is no lower side any more, then the upper half presses the leaf down, and because this pressure is constant, the leaf will not move any longer. This theoretical explanation is completely confirmed by the results of the experiment. But also the small swingings in a normal leaf, which is in a diurnal position, can be explained in this manner. The tension of the lower side of the node, for example, is then greater than that of the upper side, but quite likely not much so that, when for all kinds of physiological reasons, such as root pressure,

/61

respiration, evaporation, assimilation, the turgor of the lower side decreases somewhat, the upper side would press the leaf downwards to some extent. Then, <sup>/62</sup> when the tension again increases on the upper side, the blade again rises, etc. On the other hand, at night the pressure of the upper side is so much larger than that of the opposite side that small changes in the tension are not expressed, whereby the night peaks become quite regular.

Perhaps further investigations of the turgor in the two node halves will confirm this hypothesis.

I continued to observe the plants which served for these tests for some weeks in the greenhouse, without noticing any change in the position of leaves. They continued to look completely healthy, but P50 did not move any more, while P50' continued to move up and down around the horizontal.

As I have already mentioned in the introduction, the leafstalks were fixed in the way indicated by Pfeffer. Yet, I found such a firmly fastened stalk unnatural to a high degree. Therefore I wanted to omit further fixation. But then, of course, it had to be first established by a test that leaf blade movements are not changed thereby. Now, the movement of the stalk is opposite that of the leaf blade. The leaf droops in the evening; the stalk, however, moves upward. The movement is extremely small in comparison to the amplitude of the blade. On a strong plant, I recorded the movement of one leaf, P29, the stalk of which was fixed in the usual manner. Of the opposite leaf, P29', the movement of the leaf blade is recorded, whereas the leafstalk was free, and also recorded a curve on the kymograph with an indicator. The pull thread of it was fastened 7.5 cm from the stalk node. The stalk movement thereby could be directly compared with that of the two leafblades, where  $HD=7.5$ .

Figures 19 and 20 permit us to see the obtained results. The first one shows that the daily movement was very regular, the point of reversal always fell around 12 o'clock midnight and 12 o'clock noon, just as in the Figures 4 and 5 from the first half of November, whereas these tests were made at the end of January. Figure 20 shows that the amplitude of the blade with free stalk, it is true, is somewhat smaller, but is not deviating from the other leaf. The <sup>/63</sup> leafstalk hardly moved; between 12 and 2 o'clock in the afternoon there was a sudden drop, quickly followed by a very slow erection which, about 6 o'clock in the morning, again turns into a gradual drooping until 12 o'clock. The stalk

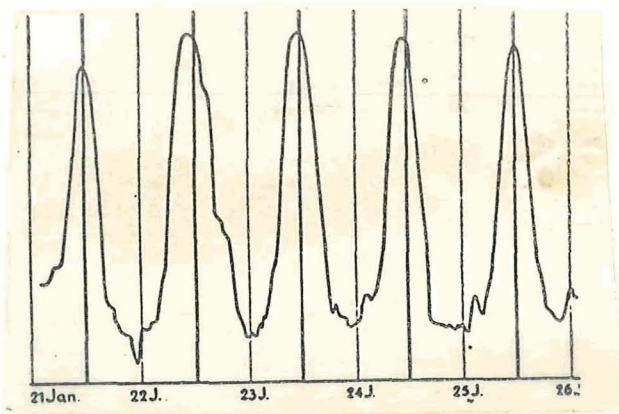


Figure 19: P29. Leafstalk fixed. Greenhouse experiment.

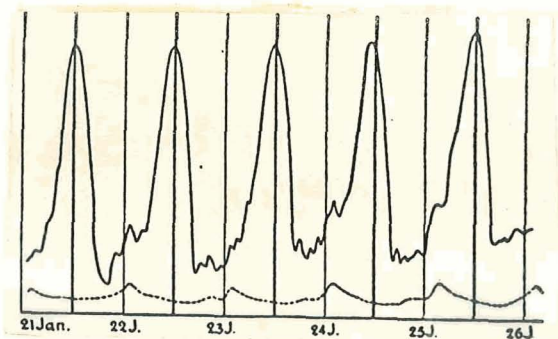


Figure 20: P29'. Solid line= leaf blade; dotted line=leafstalk. Greenhouse experiment.

is thus apparently always a few hours behind the leaf blade, and it works opposite to its movement, so that the amplitude of the blade gets smaller. As a result of this test, which I rechecked several times, I started to leave the leafstalk free in my further experiments, so that only the common stem of the plant was fastened to a stick to prevent occasional bends or pulls.

### C. Tests in the Dark Chamber

#### 1. The Movements of Plants Raised in Constant Light

The arrangement of the dark chamber was already discussed in the introduction.

Pfeffer [32] found in 1915 that *Phaseolus* leaves continue their daily movement in constant light provided that the nodes are wrapped with black cotton pads. Stoppel [47] found in 1912 that she can grow *Phaseolus* in constant darkness from seed to strong etiolated plants with good nutrition. Such seedlings still have a daily cycle. Cremer [11] confirmed this in 1922, but in his mine

/64

shaft this movement ceased. For *Calendula* flowers, too, Stoppel [45] found that they continue to make movements when raised in constant darkness. All investigators are, however, in agreement about the fact that in constant light the periodic movements soon cease, except when in *Phaseolus* the node is covered.

Of course, I also wanted to investigate the behavior of *Canavalia* in this respect. However, I was not successful in growing plants in the dark from seed. The young, yellow leaves remained extremely small and rigidly pressed to the stem so that a recording was impossible. Therefore I made a change to let them grow in constant light. Whenever light, temperature, and humidity were completely constant in the chamber, a number of seeds from Leiden were placed in a flower pot and between the two 500-watt lamps. Recording started after six weeks, when the plants had developed and become robust. The leaves were smaller than those normally grown in a greenhouse. Average length of leaf blades was 10 cm, as compared to the length of 15 cm of a normal leaf. The entire plant was longer, softer and less grown; thus, it showed all the marks of too little light during growth. Although I experimented with two lamps of 1000 candles each, at a 50 cm distance, the light intensity was apparently not sufficient for normal development of the organism. However, for nyctinasty, this lighting was satisfactory; for the amplitudes of the plants from the greenhouse maintained an even larger movement when they were periodically illuminated in the dark chamber.

As already mentioned, there is no difference between summer and winter curves. Thus, absolute light intensity is, as it seems, of no importance for the size of the movement's amplitude. For that matter, Pfeffer [32], in 1915, still used a somewhat less intense light. On page 51 under Figure 14B, he /65. mentions four tantalum lamps, each with an intensity of 25 candles!

Initially, the leaves of *Canavalia* grown in this manner appear to be peculiarly curled, but as they get older they become smoother. To my amazement, I saw that the leaves were by no means motionless, but that they had a clearly expressed daily period. The night peak fell again at about 12 o'clock at night. The leaves drooped deep in the evening, but during the day they would not stand entirely horizontal. In Figure 21 (see page 67 [of foreign text]), we find the curves which P19 and P20 described in 14 days under completely constant conditions. It is surprising that there were very many irregular movements. From 22 to 26 January, for instance, there is very little marking, whereas from the 26<sup>th</sup>



on periodicity is clearly evident. When we compare the curves of these two plants, we find more mutual differences than two such plants should have shown under normal conditions in the greenhouse, but yet the general agreement is striking. Nowhere do the movements of leaves act against each other. When one of them has a large amplitude, we find the same in the other. Sometimes the movements themselves are so alike that the drawings almost cover each other, as on 20, 27 and 28 January.

I had raised and recorded different plants in this manner, but I always obtained the same results. From 2 to 9 February, I recorded on the kymograph a plant from Leiden seed and one from Surinam seed, raised in constant light.

The Figures 22 and 23 show the curves obtained. Generally, there is much agreement, but the West Indian plant P28 had much smaller swingings than the Leiden P21. Notwithstanding the fact that this plant originated from seed which grew under such entirely different climatological conditions than the one from Leiden, the leaves show about the same periodicity as those of the Holland plant. This was also found by Stoppel [48] for *Phaseolus*. In 1916 she had compared beans from Java and from America with those grown in Germany. She could not observe any difference. Her Figures 17 and 18 of page 633 are supposed to show a difference, but are not indicative; also, too short (only 3 days). /66

The Surinam plants developed much better; indeed they were longer than normal, but the leaves were a fine dark green and not curled. The upper membrane was more shiny than in the specimens raised in the greenhouse. Generally, West Indian plants make a strong, healthy impression. The seed, for instance, is almost twice as large as the European. Yet, this only is natural, since the first has grown under favorable natural conditions; the latter, however, is always in a more or less abnormal greenhouse atmosphere. It comes to mind that the West Indian plants, because of their much stronger nature, should also respond much sooner to all kinds of external and internal influence, and thereby they should show many more swings.

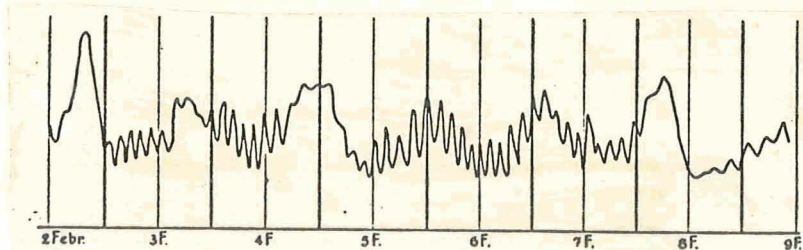


Figure 22: P28. Raised in constant light from Surinam seed. Dark chamber.

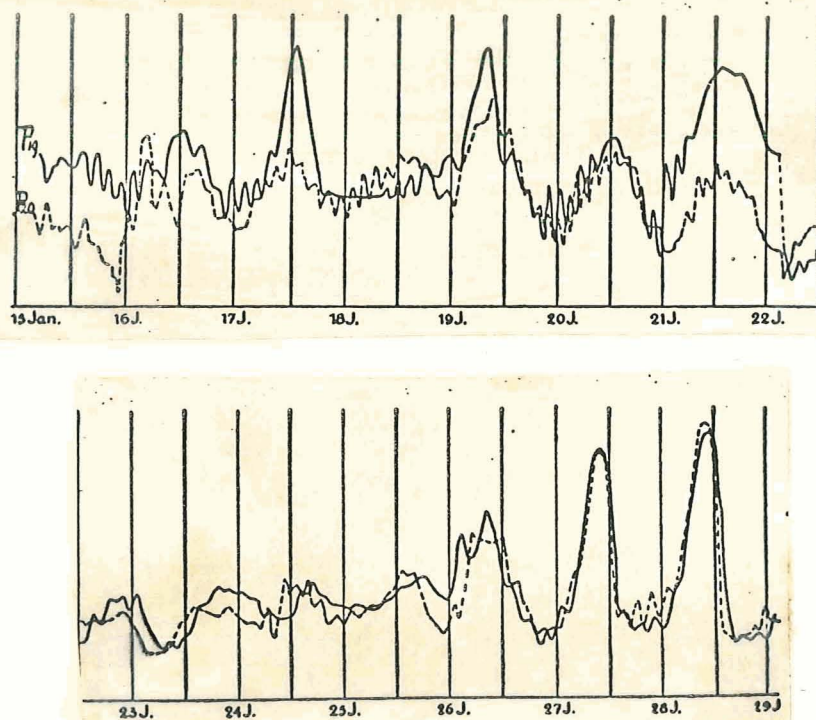


Figure 21. P19 (solid line). P20 (dotted line). Raised in constant light. Dark chamber.

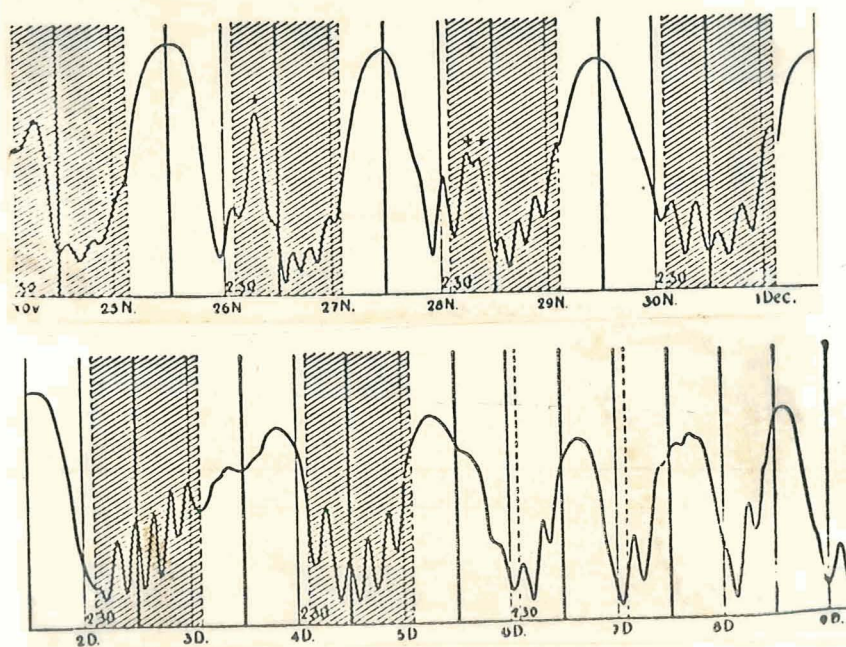


Figure 24: P6. First, 24-hour period of light. and 24-hour period of darkness; then, constant light. Dark chamber.



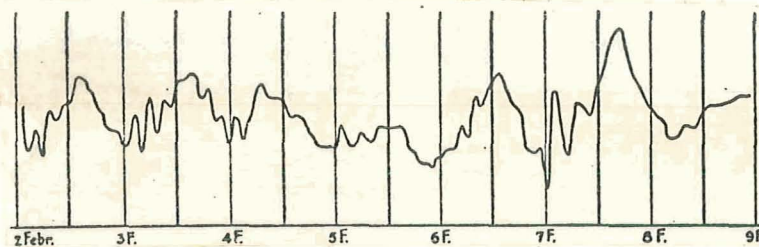


Figure 23: P21. Raised in constant light from Leiden seed.  
Dark chamber.

"Autonomous or not," that is the great problem for which I seek a decisive proof. The remarkable fact that plants, which up to then had nothing to do with any known periodicity in their life, still exhibited approximately normal daily movements in constant light, is a point in favor of an ability of movement established by heredity, and thus autonomous in a 24-hour rhythm. That the movements in constant light are not so particularly regular, has its cause in a second, let me say temporarily "harmful," action of constant light, to which I shall return later, and this seems to counteract the regular continuation of sleep movements.

Against this argument for autonomy speaks the fact that every time the two plants described chiefly the same curves on the drum, even when the two plants were of totally different origin. It seems as if the same outside stimulus which in itself must also be variable, is the direct cause of the movements. The possibility exists, however, that all known factors are kept completely constant, so that we are again forced to take Stoppel's hypothesis about the conductive ability of the air into consideration. The sharp criticism of Schweidler [37] and Sperlich and Cremer [11] warns, nevertheless, to extreme caution. Therefore, I shall rather speak for the time being of an unknown factor which I shall call X for the sake of convenience. We will deal with the possibilities existing for X in the last chapter.

## 2. Introduction of a 24-Hour Period of Light and a 24-Hour Period of Darkness

I then decided to find out what influence another period would have upon these leaves. I did not have an opportunity to induce a short period of 6 hours light and 6 hours dark, for instance. Therefore, I chose 24-hour light and 24-hour darkness periods. Plants P6 and P8, raised under constant conditions in constant light, had almost normal movements. On 24 November,



I switched off the lamps in the afternoon about 2:30; next day, they were again turned on at the same hour, and so forth. Figure 24 shows the result. The following day the leaf drooped much lower during the light period, with the reversal point still falling about 12 o'clock, that is, at midnight. On 26 November there is still a rather deep fall of the leaf in the evening, indicated by an asterisk (\*) in the figure. But immediately thereafter, a very rapid erection follows, so that still during the same night, around 2 o'clock, /69 the leaf reached a particularly high position. As the figure shows, this persisted during the darkness period. Two days later, after a deep position was reached in the light again, we still find an indication of a night peak about 6 o'clock in the evening, indicated in the figure by two asterisks (\*\*). Then the movement continued entirely normally. After 48 hours a "night peak" occurred in the light, and in the darkness, when the leaf stood in the highest position, there were many small swings. The course of these curves was almost alike in the two plants. I was unable to determine the cause of the irregular movement on 3 and 4 December. These curves show that the new period is assumed immediately, without important transitional forms. This certainly shows the great influence which light exerts on sleep movements. The peculiar thing is, however, that the lowest position occurs in the light period, and its reversal point is always around midnight. Thus, the highest position occurs in the period of darkness. As was already mentioned at the beginning, the small swings occur now during the period of darkness, when the leaf is in its highest position, from which we can again conclude that they are a result of it. From December on, the light was kept on and that same night the leaf reached the lowest position, instead of remaining in a diurnal position. The figure shows that the course of the curve continues entirely normally. There is nowhere anything of a 24 hour period to be noticed. The easy induction of the new period should lead us to conclude that, as was said before, alternation of light and darkness is the immediate cause of movements, but when we then see that normal curves immediately occur again in the constant light, we are again inclined to believe in autonomy or in "electricity." I would like to interpret the phenomenon as follows. Factor X, which causes development of periodic movements in constant light, is also present during artificial illumination. Thereby, with (artificial) alternation of light and darkness, we get an image of movement in the dark chamber which is perfectly identical to that of the leaves in the greenhouse. When

then we start to give 24 hours of light and 24 hours of darkness, then consequently this might say: 24 hours X certainly, and 24 hours not. If the light continues constantly, then there is only the one factor, which I will call "natural factor" X, that immediately calls for the appearance of the normal curve; for the X of the light still works, it is true, but now constantly. It might also be the case that factor X, in addition to light, continues to carry on its influence. In other words, the light and factor X have a similar effect on the leaf. I will return to this subject on pages 82 and 95 [of foreign text]. Because of the already mentioned occurring harmful action of constant light, perhaps also through X itself, the movement becomes less regular in constant light. However, I cannot as yet explain why the lowest position of the leaf always occurs in the light period, and the reverse point after about 12 hours. During all this time the plants were entirely healthy. In 1907, Pfeffer [29] had also induced a 24 hour light and 24 hour dark period in the leaflets of *Albizzia lophanta*, with good results. However, he did not make this experiment with *Phaseolus*; he had introduced a period of 18 hours light and darkness, and then found complete adjustment of the leaf to this light change. From his Figure 25A and B we can see that the lowest position of the leaf occurred in the light period, but the highest position in the 18 hour darkness. During this time the leaf carried out numerous swings, just as I had found in *Canavalia*. He does not mention at all that the highest position occurs in the period of darkness; it seems he could find no explanation for it. When he thereafter kept the leaves in constant light, the leaf movements soon passed into a normal rhythm of 12 hours, and finally stopped entirely, as always in constant light. Here also, we have the same result as in *Canavalia*, with the only difference that the latter does not cease moving in the light. /70

### 3. Tests with Stems

Before continuing the discussion of tests in the dark chamber, I would like to report some remarkable results which I had obtained in the meantime in the experimental greenhouse. It seemed that cut leaves continued their sleep movements for some time. I asked myself whether perhaps the leaf blade could be eliminated so that the node would carry out the movements entirely alone, either autonomously or as a result of one or another stimulus. For this purpose, I removed all the leaf pulp and four ribs from the cut leaves, so /71

that solely the node with the midrib remained. The pull thread was fastened to the rib at the usual distance of 7.5 cm. This entity (leaf stalk, midrib and node) I will call "the stem," for the sake of brevity.

I placed the stem in the manner of cut leaves in bottles, and checked the movements. Indeed, the movement continued, even though much weaker than in normal plants, which is not surprising after such maltreatment. It was possible that evaporation through the midrib would play a certain role, although this

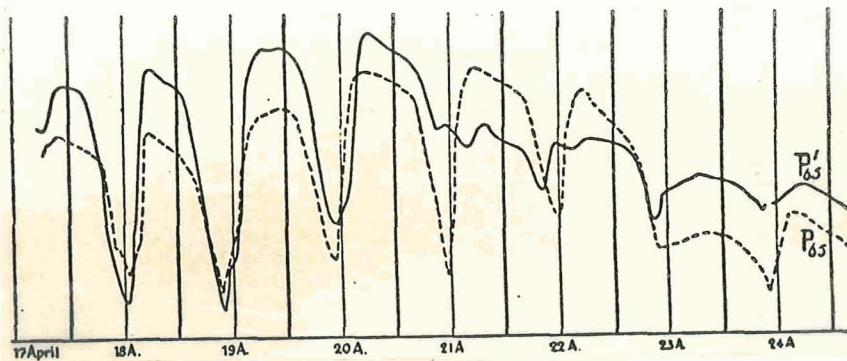


Figure 25: P65' (solid line). Stem. P65 (dotted line). Stem with paraffin covering. Greenhouse experiment.

did not seem probable, because a layer of wound cork was supposed to form quickly. I smeared one stem with a layer of paraffin having a low melting point ( $45^{\circ}\text{C}$ ), so that only the node remained free; the other served as control; in addition, the leaf of a whole plant was also present for comparison. In Figure 25 we find the curves of P65' without paraffin and P65 with paraffin. Definite differences cannot be observed; certainly one of them has somewhat larger amplitudes than the other, but a distinct agreement is apparent. As of 23 April, amplitude dropped suddenly in both, without, however, undergoing perceptible /72 external changes. The nodes looked completely green and healthy, and turgescence did decrease. The fact that such stems still showed vigorous movement after five days is remarkable. Despite the tremendous wound stimuli, the node did not lose its reaction capacity. Amplitude of movements was larger during the first days than that of a cut leaf. I shall return to this, and also to its cause. Since the leaf blade is no longer present, the small swings in the daylight have disappeared, which is again proof of the fact that these were caused by the blade's weight, and because the tension of the lower side of

the node carries out small oscillations under the influence of internal processes. The stems reach their deepest position about 6 o'clock in the morning, and then return to the diurnal position much slower than normal leaves, so that the night peaks become much wider.

From this figure we also see that the third and fourth peaks have maximum values, followed by a rapid decrease. An explanation of this phenomenon, which we will encounter several more times, is probably that because of the violent wound stimulus after cutting the nodes are less active; then, gradually, the complete reaction capacity returns, but now the withering process should make itself felt more and more, whereby the amplitudes will soon decrease in size. It is therefore not desirable to experiment with such stems longer than 5 to 6 days.

I went still further. I cut the leaf stalk off stems to such an extent that there was about 1 cm left for fastening in the glass tube of the bottle. Everything was also cut off the midrib, so that only 1/2 cm was left at most. To this rib piece I fastened with paraffin a thin capillary tube, 1 mm thick and 9 cm long. The pull thread was fastened to this glass rod at the usual distance of 7.5 cm. Yet, the movement did not cease, as we see from Figure 26 for P75 and P81. During the first days, the movement was quite considerable; then it decreased rapidly in size, which should be expected after inflicting such a wound.

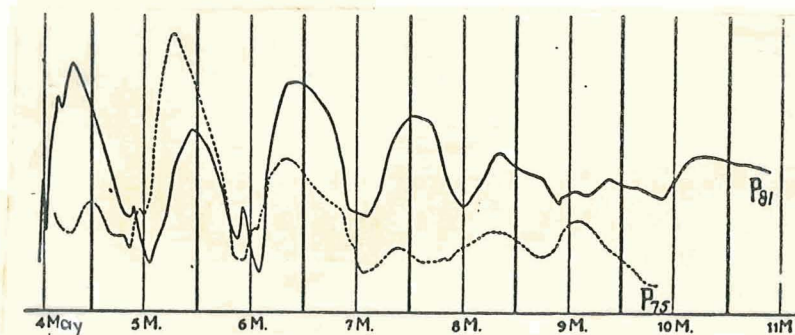


Figure 26: P75 (dotted line), leaf blade node + glass rod; P81 (solid line), the same. (Greenhouse experiment.)

These tests showed that not only the cut leaf, but also the stem and almost the node by itself, was able to carry out periodic movements for a few days. Therefore, it is the node which perceives the stimulus, the periodically changing



X, for example, and, as the result of this, experiences a change in turgor on the lower side, whereby the leaf blade is moved upward or downward. The other living functions of the plant, such as respiration, root pressure, evaporation and assimilation, obtain as a result of a much smaller significance in nyctinasty, although it is certainly probable that they could exercise some influence on it. The nodes have always a dark green color, so that it is not impossible that assimilation, which of course should continue in it, influences the movement of stems. To investigate this, I took two stems, P62 and P63 (from Surinam seed), and put black cotton around the node of the latter so that all sources of light were excluded and the movement could continue. It was to be expected that amplitudes would be reduced in size since there existed some resistance caused by the cotton. The weather was particularly dark and thus less suitable to make a comparison on assimilation. I therefore made this test in the dark chamber where temperature and humidity were constant, and the plants received light from 8 o'clock in the morning to 8 o'clock in the evening, while they stood in darkness during the other 12 hours. In Figure 27 we find the obtained

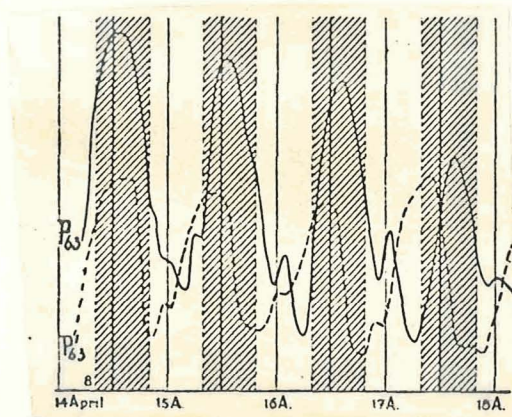


Figure 27: P63' (dotted line) stem; node covered with black cotton. P 63 (solid line) stem; non-covered node. Dark chamber.

curves from which it seems that, it is true, P63' had a less large amplitude; however, we had expected that. Otherwise, this stem, in spite of the black cotton, had an entirely distinct sleep movement for almost 5 days. I repeated this test a few more times, and always obtained similar results; from this we can conclude that assimilation has little or no significance for the development of movements.

As can be seen from the figure, movements in the dark chamber were much more regular and also greater than in the greenhouse. According to all probability,

this is due to the greater humidity in the dark chamber; this is corroborated by the following observation. From a lot of young (about three week old) plants, many had extremely weak leaves and stalks.

The leaves were magnificent in size and color, but during the daytime, when it became warm in the greenhouse, the leaf blades started to droop, not because the node caused it, but by the reduction in the turgor in the blade pulp. The ground in which the plants were was quite wet; I therefore ascribed the limp condition to the dryness of atmosphere, assuming that more water evaporated from the leaf blade than was absorbed through the roots. However, the greenhouse was thoroughly sprayed twice daily. I took a number of plants with me to the dark chamber, where there was just a normal periodic illumination for the running tests. The next day the plants had already almost recovered, and on the following day they were recovered enough that I could use them for tests. I did this frequently with such flaccid plants. Accidentally, however, I discovered that the roots of such plants looked brownish and sick. I sent some of the plants to Prof. Dr. Joh. Westerdijk in Baarn, who was kind enough to tell me that this was a "foot disease" caused by a *Fusarium*. It was explained that evaporation and water access were not in balance during the day. Of course, I no longer used such plants. /75

#### 4. Reversal of the Normal Period

On page 68 we saw that it is easy to induce a 24 hour period in the leaves. It was to be expected that it would be equally easy to reverse the period, to make darkness during the day and to give light at night. In 1907, Pfeffer [29] had already reported that this is not difficult with *Phaseolus*. I made my tests with a large number of plants, as well as with cut leaves. I shall first treat the latter ones, because I wish to discuss another problem at the same time as the discussion of the results with the whole plants.

I took four different plants, raised from Surinam seed in the greenhouse, and two grown in the dark chamber at constant temperature, humidity, and constant light (also from seeds of West India). Of each of these four plants, I took a leaf and put it in the usual manner in a small bottle on the kymograph in the dark chamber. On 2 March, about 4 o'clock in the afternoon, I made it dark; about 8 o'clock in the evening the lamps were turned on again. The next morning at 8 o'clock the light went out again, and so forth. In Figure 28 we see the

curves of P<sub>42</sub> (raised in constant light) and P<sub>41</sub> (from the greenhouse).

Although P<sub>42</sub> came into darkness for the first time, it did not react differently from the one from the greenhouse.

I again call attention to the fact that the heavy time-line which now falls in the light period is the true night and the thin line where the date is indicated is the true daylight time; here it falls right in the period of darkness. It seems that the first 24 hours cause some disturbances in the movement. But by the 3rd of March, about 8 o'clock in the morning, the reversal of the leaves had already started, so that 24 hours later the movement was regular and reversed. On 4, 5 and 7 March, at approximately 6 o'clock in the morning, P<sub>41</sub> shows a faint tendency to carry out an ascending movement; however, this could not be pushed through. In all leaves, the point of reversal was about 12 o'clock noon and after 12 hours at night; we observed this repeatedly. Regardless, we see from these tests that cut leaves reverse their period immediately, when they are forced to this by another illumination.

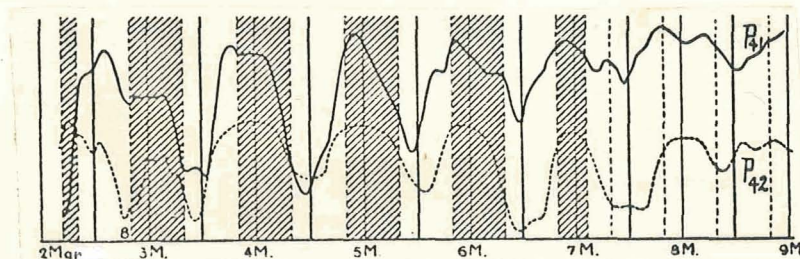


Figure 28: P<sub>41</sub> (solid line) raised in the greenhouse. P<sub>42</sub> cut leaves (dotted line) raised in constant light. Dark chamber. Reversed period.

We should now conduct the same experiments with whole plants. Preliminary tests had already shown that it was possible to revert the period of a whole plant without any difficulty, but I now wanted to find answers for the following questions:

A) Is the reverse period accepted more quickly when, after a few days of normal light and darkness, the reversal starts with a) the non-lighting of the light in the morning, or, if the reversal starts with b) non-extinguishing of lamps in the evening?

B) When the reverse period becomes regular after a few days, which period shall the movement revert to when the plants are subsequently kept a) in constant



darkness, or b) in constant light?

In each test it was possible to answer one part of A and one part of B; two test series were therefore necessary, each for a few weeks. For each test series, I took four strong plants raised from Surinam seeds. After I investigated these questions in Delft, Prof. Went was very willing to permit the equipment of the dark chamber of the Utrecht Botanical Laboratory to be arranged in exactly the same manner as had been done in Delft. The Utrecht Hortus has a number of *Canavalia* plants from West Indian seeds, so in August I had the opportunity to corroborate the obtained results in Utrecht with plants which grew up under other conditions.

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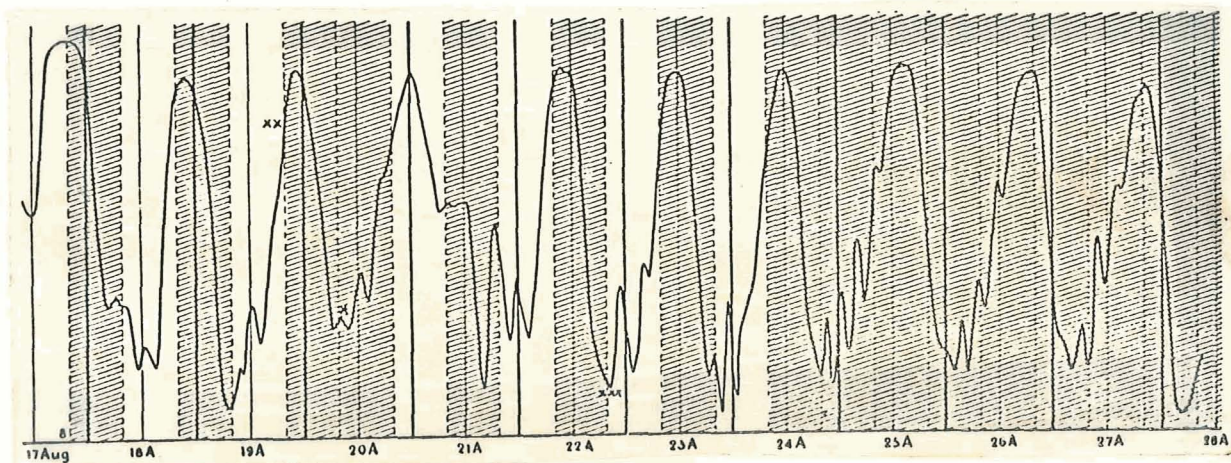


Figure 29: P121 (raised in Utrecht). Normal period; reversed period; constant darkness. Whole plant. Dark chamber (Utrecht).

It is remarkable that the curves of all the plants are so similar that I can be satisfied with reproducing one of each series. I reemphasize, however, that this is not a curve which accidentally supplied a desired result, but is one of many which all gave the same result. Thus Figure 29 shows the curve of P121 (Utrecht), transferred from the greenhouse to the dark chamber on 17 August with 3 other plants. The light went out at 8 o'clock in the evening; the next morning at 8 o'clock it was on again, and so forth. This was thus the normal period. When the amplitudes were very large and regular

/78



(20 August), the lamps were not turned on. This occurred at 8 o'clock in the evening; the periods were thereafter reversed (Question Aa). Often, in the normal curves of leaves we find a small droop about 6 o'clock in the morning, when they are almost in the highest position. This droop is directly followed, however, by a renewed ascent. We see the same here also, on the night of 19 to 20 August, indicated by an asterisk (\*). A stimulus arrives, then, with the darkness at 8 o'clock in the morning, which drives the leaf to further drooping, so that the reverse point falls exactly at midnight. On 21 August the leaf droops considerably at noon, but the turning point comes around 8 o'clock in the evening, so that now a very high position is reached at midnight in the lighted period. The reverse period then takes over; the lowest position is always assumed around noon, while the highest position is at 8 o'clock in the evening. Although the light is considerably weaker than in the greenhouses at this time of the year, the amplitude is especially large. From 24 August on, darkness remains constant (Question Ba), instead of the light's being turned on around 8 o'clock in the evening. The lowest position on the next day is therefore reached somewhat later. A day later we see that the leaf starts to go upwards around 8 o'clock in the evening; in a normal period it is still drooping about this time (see \*\*). From the 27<sup>th</sup> day on, the original period is practically back.

Before discussing miscellaneous matters, I shall discuss the second part of the questions. We find a curve of this in Figure 30, of P111 (Utrecht). Normal illumination was provided for the first two days; then on 6 August, around 8 o'clock in the evening, the light was left on; it was put out at 8 o'clock next morning, and so forth (Question Ab). We see a reaction the first night /79 the light stays on. The ascent is much slower, so that next day the highest position is not reached until 3 o'clock in the afternoon (at \*). (With normal illumination, this is reached at 8 o'clock in the morning; e.g., 5 and 6 August.) It then swings around the horizontal and starts to droop around 12 o'clock at night (normally 3 o'clock in the afternoon), so that the point of reversal now falls at 6 o'clock in the morning (at \*\*). Further movement is regularly reversed: around 8 o'clock in the evening the highest, around 12 o'clock at noon the lowest position, precisely as in the previous case.

We could certainly conclude with great assurance that the reversal of the period is accomplished equally quickly with initial light or darkness, or

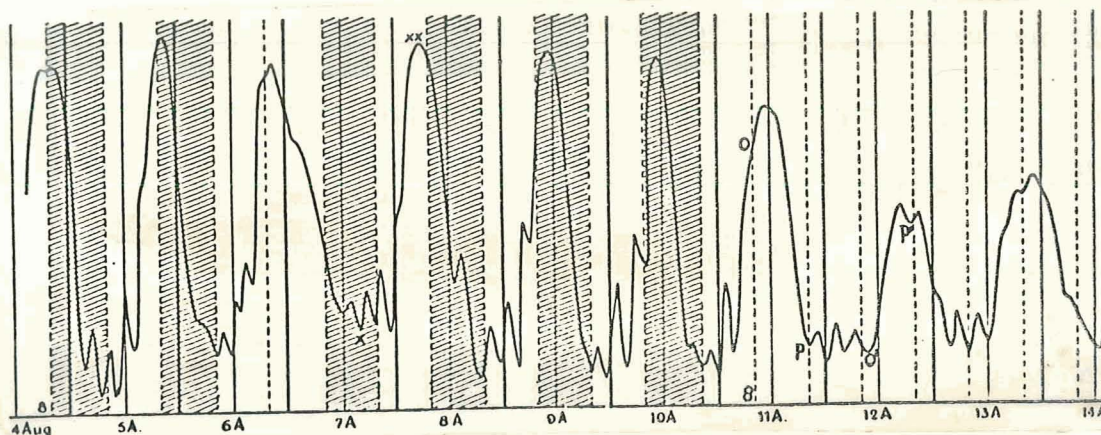


Figure 30: P111 (raised in Utrecht). Normal period; reversed period; constant light. Dark chamber (Utrecht).

even with constant darkness for the first 24 hours.

On 11 August, the light was left on at 8 o'clock in the morning, so that the plants got constant light (Question Bb). The leaf immediately reacts with a less deep drooping (all eight examined plants did so). Next morning at 8 o'clock the leaf was still in a daylight position. For a good survey of this striking reaction, we should compare the following points:

/80

11 August, 8 o'clock A.M. (O) with 12 August, 8 o'clock A.M. (O'), and  
11 August, 8 o'clock P.M. (P) with 12 August, 8 o'clock P.M. (P').

The normal period thus returned in 24 hours; nothing remained of the reversal.

The results of this test series thus become:

1. Reversal is unaffected by whether the initial period is 24 hours light or darkness.
2. The reversed movement is completely similar to the normal movement.
3. Despite the comparative weakness of the artificial light, the amplitudes are of the largest size; in the evening the leaf blade stood against the stem, and during the day it is often obliquely erect.
4. If the reversal is followed by constant darkness, the normal period returned with equally large amplitude after 3-4 days.
5. If constant light follows the reversal, after 24 hours the normal

rhythm has already returned, but with smaller amplitude.

I emphasize again that all plants, both in Delft and in Utrecht, displayed this behavior; only in constant darkness did it last a little longer before the normal period distinctly returned. Yet the curves can never convey the impression which the observer gets when he, accustomed to finding the plants in deep sleep position at 8 o'clock in the morning in case of the reversed period, sees them on the next morning standing nicely horizontal after only one extra 12 hour period of light. When constant darkness follows, the difference is more gradual. The leaves then stand obliquely in the morning, and the contrast is less striking.

In 1905, Semon [39] found somewhat similar effects for *Acacia*. When he exposed the plants to periods of 24 hour light and 24 hour darkness, and thereafter to constant light or darkness, he saw that in both cases the original normal period returned.

On the other hand, in 1907 Pfeffer [29] induced in *Phaseolus* a reversed period, but found that thereafter, in constant light or darkness, the movements continued in the new tempo, not in normal tempo, until they finally stopped. He says on page 436: "If we create darkness during the daytime and light during the night, then the phases of after-swings are also shifted by 12 hours. Since these are now running opposite to the after-swings of normal sleep /81 movements, it is clear that the after-swings are conditioned only by the induced periodicity of movement, and that, therefore, no other kind of external conditions are playing a role which would be repeated in an unchecked manner with the alternation of the day."

In 1911, Pfeffer [31] found that *Phaseolus* leaves in constant light continue their daily movements when the nodes are covered with black cotton. He first gives the leaves a reversed period, which they accept smoothly; he thereafter brings them into constant light to demonstrate that no "light rigidity" occurs. When, however, we look at the curves which he reproduced (Figure 16A and B, page 54), it seems that after three days the leaves again move in the normal manner. Thus they do not continue their reversed movements, as Pfeffer reported in 1907, and from which I quoted above. It is odd, however, that Pfeffer does not say anything about it. It seems improbable that he would not find it surprising that, for instance, on 2 November the leaf had

the lowest position at 12 o'clock noon, whereas on 5 November it stood in its highest position at the same time of the day.

We can explain the reversal of normal movements in constant light or darkness by assuming that the plant still has an autonomic ability of movement, which, however, is strongly influenced by the alternation of light and darkness; in other words, the aitionastic reaction is stronger than the autonomic reaction. Yet, if the first is not present, the second will appear, as in constant light or darkness. However, we can equally well say that Factor X, which, we are assuming for the present, is markedly representative in artificial light, causes the reversal of movements. If the plants then come into constant light or darkness, the "natural X," which always asserts itself outside by the alternation of day and night, again gets the upper hand, so that the movements again become normal.

This phenomenon can also be explained with a third possibility, that the light has the same effect on the movements as Factor X. After all, we reversed the period with changed illumination. If constant light or darkness follows, there is no periodically changing light influence any more, and thus X can again assert itself in a normal manner, so that the original period returns.

/82

It cannot be explained, however, why this is back after 24 hours in constant light, but appears only very gradually in constant darkness (see also page 78 [of foreign text]). That in constant light the amplitudes become smaller is another indication that, in one way or another, light opposes movements, as I have already mentioned. In the following tests we shall see this phenomenon occur still more strongly; I will therefore go into it later, and I will refer to pertinent analogies from the literature.

##### 5. Tests in Constant Darkness

In connection with the test results mentioned above, it seemed to be desirable to pursue the behavior of plants over a period of time in constant darkness and in constant light. I used both the whole plant and cut leaves and stems in this investigation. Figure 31 shows the curve which one of the four whole plants, P117, described during ten days. On the first days the curve is somewhat restless, but then it continues very regularly until 12 August. Then yellow spots begin to develop, whereafter the movement practically ceases. On



11 August I gave it one hour of light, which had almost no effect. The amplitudes were then the largest. On 13 August the dying process had already progressed so far that there was no longer any reaction. Since all authors, including Stoppel, Pfeffer, Semon and others, state emphatically that green plants show "darkness rigidity" after only a couple of days, it is striking that Canavalia plants continue to do well in the darkness for 8 days, and have undiminished movement. The night peaks always fall at about 12 o'clock and the highest position is around 8 o'clock in the morning. There is thus nothing of what Pfeffer calls "fading out" of the movements. We can see this clearly in Figure 32 for P116, cut leaves which I used from 5 to 15 August. During the first couple of days the movement is somewhat irregular, but from 8 August on, the curve is much more regular. The night peaks on 5 and 9 August

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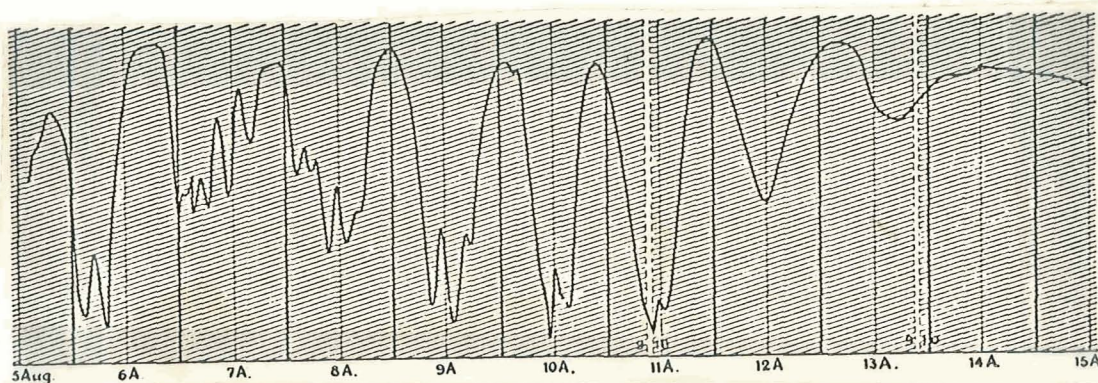


Figure 31: P117 (whole plant). Constant darkness. Dark chamber.

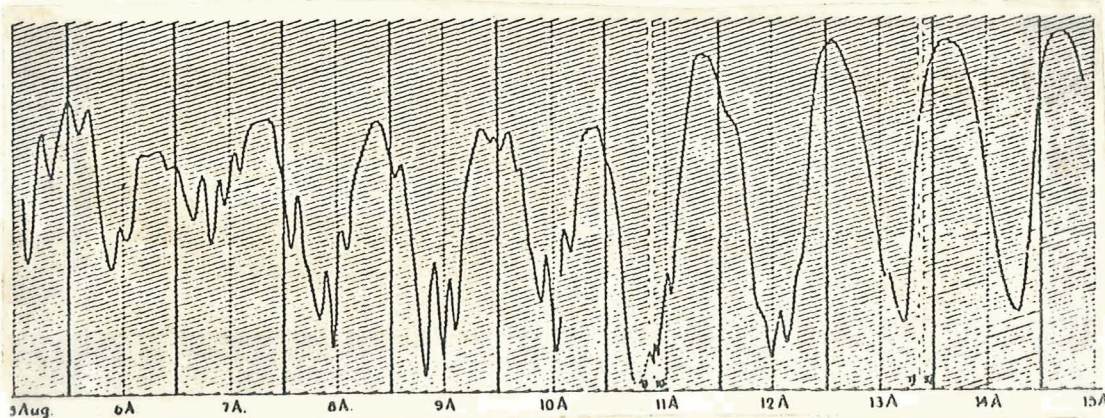


Figure 32: P116 (cut leaf). Constant darkness. Dark chamber.

are odd. I often observed this phenomenon in plants and leaves in constant darkness. I cannot give an explanation for this. The smaller amplitudes during

/84

the first days should have to do with the wound stimulus, and above all with the tremendous disturbances in metabolism, since now no assimilation takes place. On 11 August I again gave it one hour of light, whereupon a robust reaction in the cut leaves followed: the amplitudes increased considerably in size, and became less restless. Now the leaves again drooped deep, and the small swings in the night position disappeared. Although these leaves had maximum movements, the hour of light on 13 August really had no effect; but then, it was intended for whole plants, as we have already discussed. After this stay in the darkness, the leaves were still completely green and turgescient, and thus in contrast with whole plants which could not tolerate such a long stay in the darkness. Such cut leaves remained green in darkness up to 14 days.

As we have already remarked, the movements of cut leaves are not great in alternating light and darkness. This one great contrast between light alternation and constant darkness is quite clear. We find a marked example of this in the following test, during which a number of stems were kept in constant darkness. For this purpose, a sturdy leaf was made up for the stem from four different plants; this was placed in the dark chamber. To determine if the reaction capacity was in fact present, we gave both light and darkness for the first 24 hours; the next day the light was cut out permanently. Figure 33 shows the remarkable result: the amplitude increases directly and continues, almost unweakened, for six days; the movements are much larger and more regular than those of a whole plant or of a cut leaf. Nothing on this curve would indicate that we are using a stem, a node and a rib. For that matter, all four of the stems had this sort of good movement. Canavalia in constant darkness shows no trace of a "darkness rigidity," which was attributed to all other possible plants in the literature. According to Pfeffer, green Phaseolus plants are motionless in 2-3 days and die soon afterwards.

The great problem is still whether the autonomy of movements is the cause of the unweakened continuations, or if it is the often-mentioned Factor X, which by its periodic changes brings about sleep movements when "all" external conditions are apparently kept constant. I return to this in more detail in the last chapter.

In order to learn something more about the mysterious X, I made the following



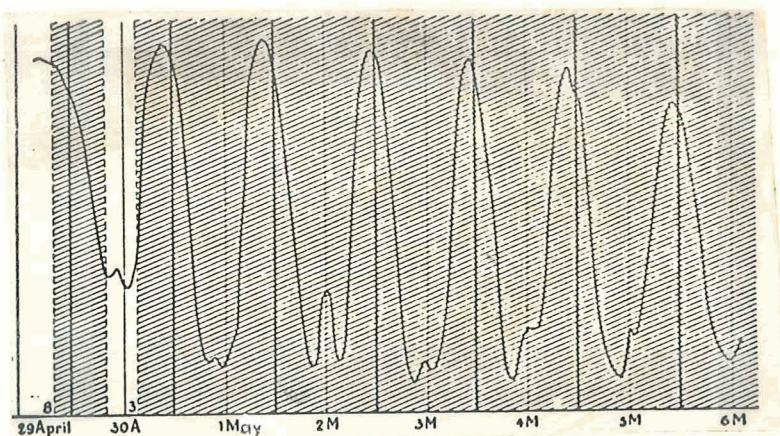


Figure 33: P80 (stem). Constant darkness. Dark chamber.

arrangement. I have already noted that I assumed that light strengthens the operation of X, or that X is in the light, so that with illumination at night and darkness in the daytime, the period can be conveniently reversed.

In the room adjoining the dark chamber, I let a 500-watt light burn under a glass bell. The glass bell was in a copper tank, closed off airtight by a rubber band. There were two openings in the tank bottom. An aquarium pump pressed air along the burning lamp (the glass bell was cooled by running water). and then through a small hole in the wall to the dark chamber (by rubber hose). Under two glass bells two cut leaves were set up, each on a kymograph, which /86 was also, of course, under the bell. The bells were standing airtight in copper tanks, which had two holes from below. Constant light was present in the dark chamber. The air, which became "light"-exposed, streamed through a long glass tube into the first bell, flew from above downwards along the plant, left this bell, and came subsequently into the second, again from above downwards along the plant, and then went outward; after this, it had to pass through a wash-bottle with water in it, so that I could conveniently check whether the pump worked.

The lamp under the bell (thus in the adjacent room) is now lighted at night and not lighted during the day. I then reasoned this way: at night the air under the bell comes in contact with X; it streams along the plants, and then with the aid of X it can reverse the movement of the leaves. I was keeping in mind the communication of Stoppel [49] in 1919 which said, on page 15: "The light's action on ion content is exactly opposite its action on conductivity.



During the dark period the absolute values of ionization are much higher and the variations are essentially higher in number than during light."

I made this test last a week long, however, and was only able to establish an indication of a reversal of the period. Circumstances prevented further work on it, so I consider this test to be very cursory.

#### 6. Tests in Constant Light

I have also pursued the behavior of plants, cut leaves and stems in constant light. I shall start with the last. Again, leaves from 4 different plants were made into stems, were checked for 24 hours in the dark chamber to see if they reacted normally, and when this seemed to be the case, were kept in constant light. Figure 34 shows the curve of P73. After cessation of periodic illumination, the amplitude increased in complete darkness; we see here, however, that in the light the movement decreased considerably. On 28 April the tips of stems started to curl; the recording was therefore no longer pure and the test was finished. Again, however, movement is not at all bad for such strongly mis-treated plant parts. /87

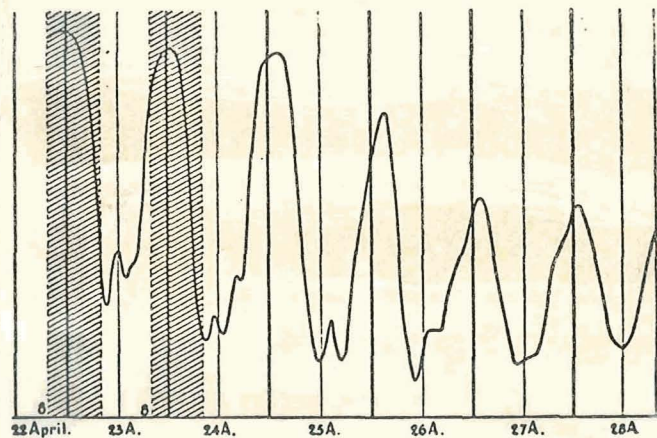


Figure 34: P73 (stem). Constant light. Dark chamber.

The curves given by each of the cut leaves present a great contrast (Figure 35). An accident prevented the movements from being recorded on the first two days; the kymographs were only useable again after 19 August. The leaves seem to have only a little movement, so "light rigidity" should occur, with

the small swings which were seen and regulated in case of *Phaseolus*. From the 20<sup>th</sup> on, however, i.e., after the leaves stood in light for 3 days, distinct daily movements began to occur again. They had a small amplitude. I shall have information on the dark period after the 24<sup>th</sup> only when we also learn about the behavior of whole plants. That the leaves had almost no movement in the first couple of days, and thereafter only a very weak movement, does not surprise us; for there are two factors present which tend to retard movement. In the first place, the often mentioned "harmful" effect of light is present; and secondly, the metabolism in the leaves will be more greatly altered by constant light than by constant darkness, since the assimilation continues in light, and thus we can suppose that the substances formed thereby should have some influence upon the turgor of nodes and should also disturb the movements. Finally, Figure 36 depicts the curve which Pl25, one of the four whole plants, described from 17 to 24 August in constant light. No recording could be made from 17 to 19 August. It is immediately striking that the amplitudes are large, very restless and irregular. This is extremely remarkable and a phenomenon which I noticed in a number of plants in constant light a few months earlier, but considered accidental; now, however, it occurred again. The figure indicates that the leaves had their highest position on 20 August toward 6 o'clock in the afternoon, whereas normally they have already almost drooped by this time. We see this phenomenon during the next days. On 23 and 24 August the leaves were still in their lowest position at noon; the night peaks made too many restless movements, also. I kept plants in constant light for different lengths of time; the movement then ran in such confusion that after a few days the leaves had a completely reversed period which nevertheless lifted them up. After some time they again became confused and passed into normal movement. Even without paying special attention to the curves, it is striking to someone who is accustomed to daily checking on the position of leaves that this is extremely uneven, even when one compares a number of simultaneously used leaves with each other. The same factors which we found at cut leaves, harmful action and disturbed metabolism, are almost certainly also the cause here. /88

From one of the preceding tests it seemed for the present that the illumination of air, before it goes over the plants, does not give a possibility to revert the period. Thus, it seemed to me not impossible that it should be the rays of light, or Factor X, which were necessary for the development of movements.

To investigate this, I did the following. One of the lamps in the dark chamber was wrapped up with a thick black photography canvas (cloth) in such a way that when the lamp burned, absolutely no light went through; the light exclusion was perfect. The other lamp was not lighted, so it was totally dark in the room. The lamp in the canvas (cloth) was switched on at night and extinguished /89

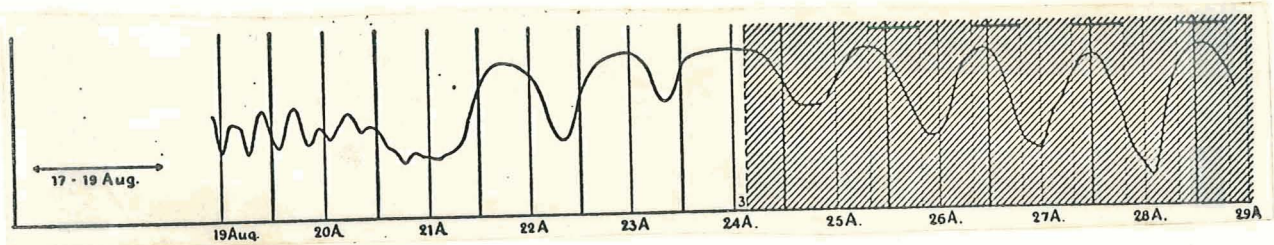


Figure 35: P124. Cut leaf; constant light; thereafter constant darkness and "illumination" with a lamp wrapped in a black cloth, in a reversed period (indicated by a line over on the figure). Dark chamber.

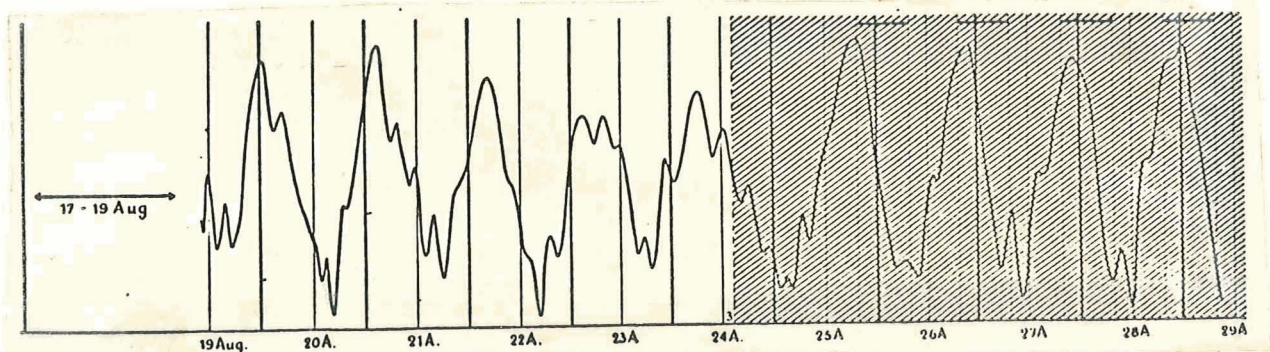


Figure 36: P125. Whole plant; constant light; thereafter constant darkness and "illumination" with a lamp wrapped in a black cloth, in a reverse period (indicated by a line over on the figure). Dark chamber.

during the day; thus, the periods were reversed. The cut leaves and whole plants which stood in constant light from 17 to 24 August thus came into the darkness now, but with an "irradiation" in a reverse period. I expected that the plants that were turned toward the lamp in black cloth would show some different sort of behavior from the plants which were turned toward the lamp that always remained unlighted. But as we can see in Figures 35 and 36, this /90

is absolutely not the case. On the contrary, the movements of cut leaves and of the leaves of whole plants are much more regular (the 12 hour "irradiation" is always indicated by a black line above on the figure). The amplitudes become larger, the night peaks again come exactly at 12 o'clock at night, and the highest position is reached before noon. The peculiar small swings in the night position disappeared; now the leaves droop deeper again. We thus must conclude that the cause of sleep movements present in the light cannot be suppressed by a photography cloth. Moreover, these tests clearly reveal that constant darkness helps regular movement, even when the rhythm is put into confusion by constant light.

In the literature we find many indications that other investigators also recognized the peculiar influence of constant light.

In 1907, Pfeffer [29] says, on page 469: "Otherwise, our research brought further examples that darkening does not produce the reverse physiological effect of illumination."

Moreover, he remarks (page 301): "In tantalum light, the plants in daily periodic light change better than in continuous illumination. A similar relationship, at least for some plants, seems to be demonstrated from the experiments of Bonnier in arc light."

And Semon [41] in 1908 mentions on page 239: "My tender seedlings evidently tolerated a continuous illumination much worse than an alternation of illumination and darkening."

In 1915, Pfeffer [32] found that leaf movements for *Phaseolus* continue normally even in constant light, when at least the nodes were wrapped in black cotton. He emphasized that the movements in constant light were practically similar to the amplitudes of leaves at periodic illumination, not to mention small deviations. /91

But when we see his curves, we see the situation to be somewhat different. Under normal conditions, the night position in *Phaseolus* falls at about 12 o'clock at night, and the highest position is between 6 and 12 o'clock in the forenoon. On page 50, he gives the curve which such a leaf, with a wrapped up node, described in constant light during six days. After three days the period apparently returned, for now the highest position falls about 12 o'clock at night, the



lowest at noon. Pfeffer does not make special mention of it, but I cannot imagine that it should not have surprised him. He does say that between two leaves of the same plant there could be great differences, when the movements of the two are recorded. During 14 days he followed up the time which occurs between two night peaks, and finds that this varies between 18 and 26 hours. In every case he shows it as a matter of secondary importance; for the chief question is that the leaf makes movements in constant light, which "keep approximately a day's periodic rhythm, from which we have the impression that this comes out more often somewhat shorter than 24 hours."

I consider this matter somewhat differently. In constant darkness the movements of the leaves of a whole plant, of cut leaves, and of stems, continue with very great regularity; in constant light they become weaker and revert entirely. From this it follows that light acts greatly as an inhibition upon the normal course of sleep movements, whether they arise by some Factor X or entirely autonomously.

## CHAPTER III

### Theoretical Considerations

We should now attempt to set up a working hypothesis which avoids contradicting the facts in the literature and which incorporates the findings which I obtained in my tests. /92

When we again question whether sleep movements are autonomous with light acting in a regulatory manner, or are caused by an outside stimulus, we cannot deny that there are findings available for both possibilities.

It seemed in section 3 that the lamina (blade) is of no direct importance in the development of movements; for when only the midrib remains, strong movements are carried out for six days longer. Also when the blade was wrapped in paper, no standstill occurred (page 48 [of foreign text]).

Assuming the second possibility, the node is both a perceptive and a reactive organ. It is improbable that the leaf stalk or dried up midrib should perceive the stimulus. For this matter, when we almost cut away these two (page 72 [of foreign text]), the movements still continue.

We must therefore consider the node to be the mechanism which, independently from the other parts of the plant, takes care of the development of sleep movements, either autonomously or aitiogenously. The expression "independently" does not imply that there is no connection between nyctinasty and other vital processes in the plant, but rather that the influence is indirect. The structure of the node is such that under certain conditions it is able to take up water in the cells, or to release it, whereby a stretching or curving occurs which brings the leaf into daytime or nighttime position. Since the node is such a specifically built organ, we are inclined to consider the ability of movement /93 in a daily rhythm to be inherited also. According to plant species or plant organ where the node is located, it will now move the leaves in the evening upwards, then again downwards; or even the leaf stalk or the leaflets go upwards, and fold up against each other.

There are different phenomena which show periodicity whose cause we do not know.

Thus, in 1915, Karsten could demonstrate [22] that nuclear divisions in above-ground plant parts had a daily periodicity. Around 4 o'clock in the morning was the peak number. Stalfelt [42] confirmed this in 1919.

In 1918, Lars-Gunnar Romell [34] established, in agreement with Baranetzki [1], that root pressure has a daily period in many plants, even when plants are raised under entirely constant conditions.

In 1925, a communication of Suessenguth [52] concerns the strongly periodic blooming period of a number of bamboo types. *Phyllostachys puberula* in Japan blooms once every 60 years. All specimens then bloom simultaneously. It is peculiar that the different blooming periods coincide with the periods of peak sunspots.

Somewhat similar findings were obtained in 1915 by Went [54] and Rutgers for the blooming time of an orchid, *Dendrobium crumenatum*. The authors believed that this must be ascribed to atmospheric temperature and humidity. For the present, according to a letter received from India, the cooling off caused by heavy rains must be considered as a direct cause.

It was also successful to bring plants to blooming by artificial cooling off.

It is then unexplainable, however, that a blooming period in Utrecht coincided with that in Bonn and Hamburg.

I gave here a few obvious examples of periodicity; many more could be listed.

The fact that in constant light and constant darkness the movements continue with the same rhythm is another point in favor of an autonomous ability of movement in the nodes. Even when plants were raised entirely in the light, they have a clearly expressed movement tempo.

The light quantity is evidently not of great importance; Semon [40] used a 10-candle lamp, Pfeffer [29] used a 40-candle to 200-candle lamp (1907, page 74), and I have two lamps of 1000 candles. For that matter, the fact that the summer and winter curves showed no differences also points in this direction.

/94



Moreover, we have seen that when, after a period of 24 hours of light and 24 hours of darkness, the plants come into constant light, normal sleep movements are carried out very soon (page 69 [of foreign text]). The same is true when, after the period is reversed, we bring the plants in constant light or darkness; the usual rhythm also returns directly then (pages 78 and 80 [of foreign text]).

We can conclude from these tests that the cause of nyctinasty is to be sought in an external stimulus. It was always easy to induce a rhythm of 24 hours; by an illumination at night and darkness in daytime, the period of leaf movements is reversed in 24 hours. Thus, the nodes are indeed very sensitive to changes caused by modified illumination. Because such small light intensities as used by Semon and Pfeffer reversed the entire rhythm of movement of all sorts of plants, we are inclined to search for quite a weak stimulus for the normal daily movements of leaves.

Nyctinasty is not a direct consequence of the alternation of day and night; for in constant light and constant darkness unweakened movements are still carried out by *Canavalia* leaves; Stoppel, Pfeffer and Cremer found the same for *Phaseolus*. Only there, in constant light, the nodes must have been wrapped in black cotton, and the *Phaseolus* plants must have been raised in darkness from seed on; thus they had to be etiolated; otherwise "light or darkness rigidity" will occur. We thus come to the conclusion that the nodes of plants with periodic movements are built from a tissue which is extremely sensitive to the daily, periodic changes of a still unknown factor, which we call X for the present. By the influence of this X, turgor changes which result in a definite movement specific for plant or organ develop in the node.

/95

If we had to count with the very great influence which light has upon the rhythm of movement, then we should assume that there are three possibilities:

A) Factor X is strongly represented in the artificial light used so it can temporarily suppress the normally changing X which causes the daily sleep movements.

B) The influence of light upon leaf movements is the same as that of Factor X, so that both are supposed to act next to each other.

C) The light has such an influence upon the node that illumination makes

its sensitiveness for X increase or decrease.

If we assume the first possibility, then the fact that it is so easy to modify the rhythm of leaf movements by a change in illumination is easy to understand. When the light is on at night, Factor X should influence the node with greater intensity than during daytime with "normal X," whereby the leaves should carry out reverse movements.

In constant light and constant darkness, "normal X" should cause the process of sleep movements; for then X is present in the light, constant or not. Hence, this does not explain why in the first case the movements were so much more regular and why in *Phaseolus black cotton* must be around the nodes. Such black cotton and paper must thus be no obstacle for X, since the movements are not disturbed by it (pages 73 and 47 [of foreign text]). In contrast, it is impossible to revert the period by "lighting" at night with a lamp which is enveloped entirely in black cloth (page 88 [of foreign text]); thus, X apparently does not go through. As to the second case of (B), when we assume that X and the light exercise the same influence upon the nodes next to each other, then we must also assume that each of them should be then able to produce a maximum result (condensation) of the leaves.

In constant light or constant darkness the movements continue without weakening. The cause of this must be X.

/96

But as we illuminate at night and keep darkness during the day, the leaf movements revert completely, while their amplitude remains equally large. This reversal was brought about by light. When X and the light exercise an approximately equally strong influence, with the reversal of movements the amplitude should become smaller, because the two factors together oppose each other.

The possibility which then remains is that artificial light (even though it is very weak, as in the case of *Semon*) has a much stronger influence than X. This seems to be rather unlikely.

When we now consider the third (C) possibility, then the differences are of such a nature that we must assume that light considerably elevates the sensitiveness of nodes for the influence of X.

We cannot as yet speculate about what X is.

When lighting is reversed, nodes are most sensitive at night, and least sensitive in the daytime; again the easy reversal of the rhythm is explained. If the leaves thereafter come into constant darkness (page 78 [of foreign text]), then the sensitiveness of nodes should indeed not be so very large, but, since the "normal X" makes itself felt naturally in constant darkness also, the sleep movements should be regularly carried on, but with small swings. If, on the other hand, after the reversal of a period the leaves come in constant light, then the sensitiveness of nodes should increase greatly, so that "normal X" can immediately express itself with full intensity, whereby within 24 hours the normal movements return (page 80 [of foreign text]). We have seen a similar result after a 24 hour period was introduced. Then, also, in constant light the normal period returned (page 69 [of foreign text]). Pfeffer [29] found in 1907 in *Phaseolus* that after a period of 18:18 of 6:6, the "night swings" went over into a rhythm of 12:12.

We could ascribe the irregularity of movements in constant light to an oversensitive, almost pathological condition of the nodes as a result of the persistent light, whereby a normal reacting ability is disturbed. Movements then become restless and confused, while in *Phaseolus*, *Minosa*, *Acacia*, et al., it is followed by "light rigidity." If this overstimulated condition of the nodes is not soon interrupted, the normal reaction ability returns. In my tests, for instance, on page 90 [of foreign text], Figures 35 and 36 clearly reveal this when the leaves come in darkness during the last days. Pfeffer calls attention repeatedly, in 1905 and 1915, to the fact that, although the sleep movements stop entirely in constant light, the leaves still remain perfectly "able to act."

If the nodes in the leaves in question were surrounded with black cotton, then the daily movements became much more normal (Pfeffer, 1911 and 1915). Meanwhile, we have already discussed the fact that the movements performed then still do not agree with the "normal curves," although Pfeffer says little about it. It seems to me that we must seek its cause in the fact that the black cottons around the nodes are a rather conclusive protection against the influence of constant light, but not entirely, so that the curves should still have an irregular course. *Canavalia* is a much stronger plant, so that also the nodes are less sensitive for the presented harmful influence of long-lasting illumination than is the case with *Phaseolus*. We can say approximately that the non-covered

nodes of *Canavalia* in constant light are in the same condition as *Phaseolus* nodes surrounded with black cotton. The difference between the different plants is thus gradual, a result of more or less sensitive nodes.

It follows from this that rigidity by continued light ("light rigidity") or by continued darkness ("darkness rigidity") in the *Phaseolus*, and others, are mutually different in value. "Light rigidity" is always a result of oversensitiveness, and abnormal condition of the node tissue, caused by too large and too continuous a light stimulus. On the contrary, "darkness rigidity" is a necrobiotic process brought about by the powerful metabolic disorders of the leaf and the whole plant; when in constant darkness the assimilation stops. Most plants cannot do anything against it, whereby dying starts rather quickly. /98 This has thus nothing to do with a special condition of the node.

The very sturdy *Canavalia* can tolerate darkness very well (see page 84 [of foreign text]), but most plants, which then also seem to be weaker in other respects, are thereby ruined. This is corroborated by the fact that Stoppel [47] in 1912, and later Cremer [11] in 1923, could raise *Phaseolus* plants in complete darkness, provided that the plants were well fertilized. Such etiolated plants do not languish in constant darkness, and thereby they continue to perform normal sleep movements.

In this connection, it is also peculiar that *Canavalia* remains green for 14 days in the dark. As soon as the leaf starts to get a few yellow spots, however, the amplitude of the movements drops considerably. Sometimes the spots occur only 24 hours after the beginning of darkness; mobility almost disappeared.

Not only is it as yet unknown what X is, but we know even less why it changes periodicity every day. Now, it seems to me not unlikely that it is the alternation of day and night which causes this periodicity. By lighting during the day, then, the intensity of X should increase or decrease. Finally, nyctinastic movements should also develop by the change of night and day, but now in an indirect way.

When X is one or another factor in the atmosphere, it is to be understood that its daily changes must be made felt also interiorly, in dark chambers, so that in continued light or darkness, with all conditions constant, the periodic changes of this unknown make sleep movements of the leaves continue.

Cremer [11] in 1923 found, remarkably enough, that in a mine shaft all movements of leaves ceased. If he brought them into the dark chamber afterwards, under corresponding conditions, then the movements returned and reversed. /99 This has all the appearances of indicating that in the mine shaft a factor was missing which is always present above the ground.

In summary, we can say, not as an explanation but as a working hypothesis, that there is an unknown factor X in the atmosphere which undergoes changes in a daily period, perhaps as a result of the illumination during the day and the darkness at night. Different plants have specially arranged organs, the nodes, which are extremely sensitive to the mentioned changes of X, causing turgor differences in the node which bring a leaf or a leaf stalk or leaflet into a desired position.

By light, the sensitiveness of nodes considerably increases; finally an abnormal condition develops which makes the movements irregular, or brings them to a stop.

The small swings in the daytime position are no direct result of external stimuli, for they occur only when the leaf stands horizontally; they also occur even if it is dark, for instance during a 24 hour period.

The static momentum of the blade with respect to the node is greatest when the leaf stands horizontally.

It is then when small changes in the turgor of the node's lower side (caused by metabolism, evaporation, etc.) should make themselves felt the strongest in the form of a more or less large up and down movement of the leaf blade.

What is X? I can be brief, since there is no single positive datum to be found. Stoppel [48-50] came to the conclusion that the conductive capacity or the ion content of the atmosphere is the cause of sleep movements, and he tried to prove this. These demonstrations are, however, rather problematic, and by the investigations of Schweidler [37] and Sperlich and Hans Crener [11] their effect was discounted, as soon as Stoppel presented them. I could never observe any influence as I arranged my plants in an isolated place, or when, with the aid of an accumulator, I let a current pass from leaves to roots, or vice versa. For that matter, the cut leaves and the stems which stood in /100

bottles with water had a normal movement, regardless of how completely isolated they were.

The whole area of electrophysiology is still so much in its starting stage and is so closely connected with the phenomena of physical chemistry that it could not be possible to set up one or another random hypotheses. As long as we did not have a single finding by experiment, however, it seems better not to launch any arbitrary theory. It is unquestionable, meanwhile, that electricity and radioactivity play a role in plant life. We see evidence of this in the many phenomena which Rosa Stoppel reported in 1916 and 1920, for instance. Since this, Kurt Stern [44] wrote, in 1924, a very comprehensive work on electrophysiology, wherein new paths were indicated for investigation. And recently (1925), Blaauw [5] and Van Heyningen have established a radium growth reaction which was caused by gamma rays. This reaction is opposed to light growth reactions, whereas from its tests it appears that its perception is also different.

Further investigations engaged in the essence of Factor X should thus move in this direction. But in further experimentation on the field of nyctinasty, we should not illuminate the whole plant with big lamps; but rather, small light bundles should fall only upon the nodes that are in question, in order that we be able to learn about the influence of different light intensities, and of different rhythms. In each case we should adduce a reversed period to plants which were standing for some time in constant darkness, and we should work with steadily decreasing light intensities. Then it will be possible to determine the light intensity whereby a reversal of the period is still just possible.

It is also of very great importance that we investigate whether light of different wavelengths has differential effects on the periods, and which light type has the supposed "harmful" influence.

It must also be more accurately determined what influence temperature, humidity, evaporation, condition of the cutaneous stomata, and assimilation have. Perhaps there should also be connection made between the occurrence of small swings in the daylight position of the leaves and certain changes of the mentioned physiological processes. /101

In addition, the investigations of Cremer in the mine shafts must be continued.

## CHAPTER IV

### Clinostat Tests

I shall finally report on the behavior of *Canavalia* plants when upside down and when revolving on the clinostat. When a plant is set down upside down, the leaf blades turn at the nodes so completely that the upper side is again facing the light as much as possible. The lower side is hence pressed against the leaf stalks, which point downward at an angle of  $45^{\circ}$ . There is no more movement; they stand unchanged all day. If they are later returned to a normal position, then after 24 hours the position of leaves and their movement is again normal.

/102

*Canavalia* therefore does not belong to the geonyc tinastic plants as distinguished by Fischer [15]; these plants, when placed upside down, continue the movements of their leaves unchanged with respect to the ground.

*Canavalia* is for the same reason not a member of the autonyc tinastic plants, which leave the movements of leaves unaltered with respect to the plant when placed upside down.

If the plants are turned about the horizontal by a clinostat, so that the stalk is along the prolongation of the axis, then after two days the leaf stalks are so revolved that they stand exactly perpendicular on the stem of the plant; the leaf blades point obliquely on the stalk, with the tips in contact with the stalk. I made both tests repeatedly in constant light, also, and always obtained the same result: 24 hours after replacement in a normal position, the position and the movements were again normal.

It was interesting that the movements of leaves stopped directly. Stoppel [48] in 1916 worked with cut leaves and found that leaves whose stalk stood perpendicular on the clinostat axis (as my plants were positioned) kept their movement only once, then in a reversed period, after which the movements became completely regular. When *Phaseolus* was set upside down, the movements continued. The leaf tips generally turned toward the ground in the morning. Hence in this case the difference between *Canavalia* and *Phaseolus* is very great. Many more tests ought therefore to be made in this field, to explain these facts.

/103



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Bot. Zeitg., 1890, 48.

Stoppel, R., "Relations of the Sleep Movements of Phaseolus Multiflorus,"  
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## REVIEW OF THE MAIN RESULTS

1. All tests were made with a single plant species: Canavalia ensiformis, D.C.

/104

2. A new method was used, whereby the recording became much more accurate.

3. When movements of several plants were recorded simultaneously, the mutual curves show much agreement, even though the plants were derived from Leiden or Surinam seeds. This indicates that an external factor (or a complex of factors) must be assumed as cause.

4. There is no difference between summer and winter curves. In addition, the curves scribed by plants in the greenhouse and plants in the dark chamber show many points of agreement.

5. When leaf blades were packed in white or black paper, the type of movements remains unchanged, though the amplitudes were somewhat smaller.

6. Cut leaves continued their movements for days, provided that the leaf stalk was removed. If it is present, the leaves will wilt after a few days.

7. Leaves kept entirely under water are immobile. They do not react to temperature differences or to a change in illumination.

8. If the upper half of the leaf blade node is removed, the leaves continue their daily movements, only with the difference that in the evening the leaf droops less, and during the day it stands obliquely upward.

After removal of the lower side of the node, the leaf droops until the point contacts the stem. Then it does not move any further.

9. It can be explained only by assuming that turgor in the upper half is very large but constant. Meanwhile, the tension of the lower side changes periodically, due to a still unknown stimulus which is called X.

/105

10. The curves of European and West Indian plants are essentially similar to each other. The latter are the strongest and able to react better.

11. If *Canavalia* is raised in constant light and at constant temperature and humidity, then the sleep movements seem to still be present. But they are not very regular and very restless. This must be ascribed to a harmful influence of light.

12. A period of 24 hours light and 24 hours dark is easy to induce. The highest position of the leaves comes then in the dark period, the lowest in the light period, in contrast with the normal curve at which the highest position falls into light, and the lowest into darkness.

13. If afterwards the leaves come into constant light, then very soon the normal period returns, but with smaller amplitudes.

14. It appeared that the stems (i.e., the leaf stalk, with the leaf blade node and only the midrib) continue periodic movements for a few days, even though the node is wrapped in black cotton. Thus, assimilation is not a necessary requirement for nyctinastic movements.

15. Leaves which are still attached to the plant, and cut leaves, could easily be brought to reverse the rhythm of their movements, so that they assume the daylight position at night, and vice versa.

16. For the course of the reversal, it is indifferent whether this happens by first keeping the light burning in the evening, or by first leaving it dark in the morning.

17. If, after the period of their movements is reversed, the leaves come into complete darkness, then after a few days the normal period returns again. If, however, this happens after reversal into constant light, then after 24 hours the original period already returns, just to get again irregular afterwards.

18. Based on these tests we came to the conclusion that most indications are in favor of an aitiogenous reacting capacity, and not of an autonomous, hereditarily established motion phenomenon.

/106

The working hypothesis which results from this is:

In the atmosphere there is a Factor X, which undergoes periodic changes each day (perhaps by the alternation of night and day).

The nodes are extremely sensitive organs in which, under the influence

of these changes in X, a change occurs in the turgor of the lower side of the node, which brings the leaf blade into night or day position.

The small swings which always occur when the leaf stands horizontally are caused by small changes in the tension of the lower side of the node, due to metabolism. In this position, the weight of leaf blade exercises the greatest influence on the node; thus, a small change in turgor results in a fairly large movement.

19. Periodic blowing of "illuminated" air along the leaves has no effect upon the movements.

20. When the lamp is wrapped in an airtight black cloth, the light no longer exercises influence on the plants; the movements cannot be reversed.

21. Leaves on plants, cut leaves and stems continue to move very regularly in constant darkness.

22. In constant light, movements become irregular, whereas the amplitudes become smaller.

23. It follows from this that, in addition to what has been said in 18 (light increases the sensitiveness for X; see 17), light harms the function of nodes more or less, whereby the course of movements becomes irregular.

24. The difference between *Phaseolus* and *Canavalia* is small. The nodes of the first are much more sensitive to light, so that "light rigidity" does develop.

Wrapping these nodes in black cotton lessens the influence of light; the movements continue more or less irregularly (Pfeffer).

Rigidity by darkness ("Dunkelstarre") is the beginning of the dying process, representing too great a metabolic disturbance. (It does not, therefore, occur in well-fed etiolated plants; Stoppel, 1912). In *Canavalia*, the disturbances are apparently much smaller; the plants continue to grow for days in the dark.

25. Tentative tests on the clinostat revealed that, when *Canavalia* plants are revolved in the prolongation of their horizontal axis, the leaves stand stiff with their tips against the stem and become motionless. The leaf stalks then stand perpendicular on the stem.

/107

26. If the plants are placed upside down, the movements cease immediately. In both cases, after replacement to normal position, the motion capacity returns in 24 hours.

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see 28).

## THESES

### I

/111

The electrolytic method is not suitable for the determination of protoplasmic permeability.

### II

Light increases protoplasmic permeability. (W. Lepeschkin, Beih. Bot. Zbl., 1909, 24).

### III

In phototropic curves, it is not permitted to entirely neglect the growth reaction on the reverse of the leaf. (Van Dillewijn, Versl. Kon. Acad. Amsterdam, 1925).

### IV

The agent of tobacco and tomato mosaicism can be cultivated in an artificial agar.

### V

For the control of blight and anthracnose of beans and other plants, trials should be made by spraying with Bordeaux mixture and other fungicides.

### VI

/112

When a specific mold occurs on a plant species, it is possible that this mold falls into a number of types which have specialized themselves on different parts of the plant.

### VII

The family tree of Compositae according to Small is the most satisfactory.

### VIII

The regulation of body temperature in insects is independent of the nervous system.

### IX

Rather than having knowledge of six plant families, it is desirable that at the final examination of secondary schools having a 5-year course, candidates are able to classify (plants).

X

It is necessary that secondary schools with modified curriculum (e.g., Lit. Econom. Division) should deal with a few subjects from General Biology.